UNIVERSITY OF ALBERTA

THE NUTRITIVE VALUE OF MICRONIZED CEREAL GRAINS AND WHEAT SHORTS FOR PIGS

BY

SUXI HUANG ©

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DEDICATION

TO MY PARENTS FOR THEIR LOVE AND EDUCATION

TO MY WIFE, SHULAN SUN, AND SON, RUI HUANG
FOR THEIR LOVE, PATIENCE AND SUPPORT

ABSTRACT

Two experiments were carried out to determine the effect of micronization on energy, starch and amino acid digestibilities in hulless barley (HB) and wheat, and another experiment to determine the nutritive value of wheat shorts and factors affecting the digestible amino acid content. In Experiment 1, micronization improved the apparent ileal digestibilities of the indispensable amino acids in HB by 5.3 to 10.0 percentage units. The differences were significant ($P < .05$) for most amino acids. Micronization also increased ($P < .05$) the ileal digestibility of starch from 79.0 to 97.3%. In Experiment 2, micronization increased the apparent ileal digestibilities of the indispensable amino acids in wheat by 2.2 to 12.2 percentage units. The differences were significant ($P < .05$) for most amino acids. Micronization also improved ($P < .05$) the ileal digestibility of starch from 93.1 to 99.3%. In Experiment 3, the digestibilities of amino acids decreased with increasing proportions of wheat bran and shorts in the wheat fractions . The digestibilities of lysine ranged from 54.7 to 64.1% and of threonine from 48.9 to 69.2%. Simple linear relationships were established between the apparent ileal digestibilities of amino acids and the neutral-detergent fiber (NDF) content in the wheat fractions. The negative correlations were significant ($P < .05$) for most amino acids. In Experiment 4, there were no differences ($P > .05$) in the ileal digestibilities of amino acids associated with NDF between the wheat fractions. The digestibilities of amino acids associated with NDF were low. Simple linear relationships were established between the apparent ileal digestibilities of amino acids and the content (%) of CP associated with NDF in the wheat fractions. The correlations were improved. In Experiment 5, the average output of mucin in ileal digesta was 12.05 g/d, ranging from 10.90

to 14.26 g/d. The average output of bacterial nitrogen in ileal digesta was 2.34 g/d, ranging from 1.82 to 2.86 g/d. There were no differences ($P > .05$) in the ileal outputs of mucin and bacterial nitrogen between pigs fed the experimental diets. There were differences ($P < .05$) in the contributions of bacterial to the total nitrogen in ileal digesta of pigs fed the experimental diets, ranging from 14.0 to 34.0%.

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TABLE OF CONTENTS

CHAPTER	PAGE
CHAPTER 1. GENERAL INTRODUCTION	1
A. Effect of Processing on Energy and Nutrient Digestibilities	1
B. Dietary Fiber and Digestion of Fiber in Pigs	7
C. Objectives of Thesis	11
E. Literature Cited	12
CHAPTER 2. EFFECT OF MICRONIZATION ON ENERGY, STARCH AND AMINO ACID DIGESTIBILITIES IN HULLESS BARLEY FOR YOUNG PIGS	19
A. Introduction	19
B. Experimental Procedures	20
C. Results and Discussion	24
D. Literature Cited	37
CHAPTER 3. EFFECT OF MICRONIZATION ON ENERGY, STARCH AND AMINO ACID DIGESTIBILITIES IN WHEAT FOR YOUNG PIGS	40
A. Introduction	40
B. Experimental Procedures	41
C. Results and Discussion	45
D. Literature Cited	59

CHAPTER		PAGE
CHAPTER 4. NUTRITIVE VALUE OF WHEAT SHORTS AND FACTORS AFFECTING THE DIGESTIBLE AMINO ACID CONTENT FED TO GROWING PIGS		64
A.	Introduction	64
B.	Experimental Procedures	65
C.	Results and Discussion	69
D.	Literature Cited	84
CHAPTER 5. ILEAL DIGESTIBILITIES OF NEUTRAL-DETERGENT FIBER, CRUDE PROTEIN AND AMINO ACIDS ASSOCIATED WITH NEUTRAL-DETERGENT FIBER IN WHEAT FRACTIONS FED TO GROWING PIGS		89
A.	Introduction	89
B.	Experimental Procedures	90
C.	Results and Discussion	92
D.	Literature Cited	103
CHAPTER 6. THE RECOVERY OF MUCIN IN ILEAL DIGESTA AND BACTERIAL NITROGEN IN ILEAL DIGESTA AND FECES OF PIGS FED DIETS DIFFERING IN THE CONTENT OF NEUTRAL-DETERGENT FIBER		107
A.	Introduction	107
B.	Experimental Procedures	108

CHAPTER		PAGE
C.	Results and Discussion	113
D.	Literature Cited	122
CHAPTER 7. GENERAL DISCUSSION AND CONCLUSIONS		128
A.	Discussion	128
B.	General Conclusions	132
C.	Literature Cited	134

LIST OF TABLES

TABLE		PAGE
2-1	Formulation (%) of the experimental diets	30
2-2	Dry matter, neutral-detergent fiber, energy, starch, crude protein and amino acid content (%) of the dietary ingredients	31
2-3	Dry matter, neutral-detergent fiber, energy, starch, crude protein and amino acid content (%) of the experimental diets	32
2-4	Apparent ileal digestibilities (%) of dry matter, energy, starch, crude protein and amino acids in the experimental diets	33
2-5	Apparent faecal digestibilities (%) of dry matter, energy, starch, crude protein and amino acids in the experimental diets	34
2-6	Disappearance of dry matter, energy, starch, crude protein and amino acids in the large intestine of pigs fed the experimental diets	35
2-7	Effect of micronization of hulless barley on the apparent ileal digestibilities (%) of starch, crude protein and amino acids	36
3-1	Formulation (%) of the experimental diets	52
3-2	Dry matter, neutral-detergent fiber, energy, starch, crude protein and amino acid content (%) of the dietary ingredients	53
3-3	Dry matter, neutral-detergent fiber, energy, starch, crude protein and amino acid content (%) of the experimental diets	54

TABLE		PAGE
3-4	Apparent ileal digestibilities (%) of dry matter, energy, starch, crude protein and amino acids in the experimental diets	55
3-5	Apparent faecal digestibilities (%) of dry matter, energy, starch, crude protein and amino acids in the experimental diets	56
3-6	Disappearance of dry matter, energy, starch, crude protein and amino acids in the large intestine of pigs fed the experimental diets	57
3-7	Effect of micronization of wheat on the apparent ileal digestibilities (%) of starch, crude protein and amino acids	58
4-1	Formulation (%) of the experimental diets	75
4-2	Chemical and amino acid composition (%) of the experimental diets	76
4-3	Chemical and amino acid composition (%) of the ingredients in the experimental diets	77
4-4	Apparent ileal digestibilities (%) of dry matter, starch, energy, crude protein, and amino acids in the experimental diets	78
4-5	Apparent fecal digestibilities (%) of dry matter, starch, energy, crude protein, and amino acids in the experimental diets	79
4-6.	Disappearance (percentage units) of dry matter, starch, energy, crude protein, and amino acids in the large intestine of pigs fed the experimental diets	80
4-7	Disappearance (grams/kilogram of dry matter intake) of dry matter, starch, energy, crude protein, and amino acids in the large	81

TABLE		PAGE
	intestine of pigs fed the experimental diets	
4-8	Apparent ileal digestibilities (%) of starch, crude protein and amino acids in the wheat fractions	82
4-9	The linear relationships between apparent ileal digestibilities of crude protein and amino acids and neutral-detergent fiber content in the wheat fractions	83
5-1	Content (%) of crude protein and amino acids in neutral-detergent fiber and the contribution (%) to the total crude protein and amino acid content in soybean meal, wheat bran and wheat shorts	98
5-2	Content (%) of crude protein and amino acids and the contribution (%) of crude protein and amino acids associated with the neutral-detergent fiber fraction to the total crude protein and amino acid content in the wheat fractions	99
5-3	Contribution (%) of crude protein and amino acids associated with neutral-detergent fiber to the total crude protein and amino acids in the ileal digesta	100
5-4	The ileal digestibilities of neutral-detergent fiber and crude protein and amino acids associated with neutral-detergent fiber in the experimental diets	101
5-5	The linear relationships between the apparent ileal digestibilities of crude protein and amino acids and crude protein associated with neutral-detergent fiber in the wheat fractions	102

TABLE		PAGE
6-1	Outputs of mucin, N-acetylglucosamine, N-acetylgalactosamine and N-acetylglucosamine/ N-acetylgalactosamine ratios in ileal digesta of pigs fed the experimental diets	120
6-2	Outputs of diaminopimelic acid and bacterial nitrogen in ileal digesta and feces and the contribution to total nitrogen of pigs fed the experimental diets	121

CHAPTER 1

GENERAL INTRODUCTION

Studies were carried out with young pigs to determine the effect of micronization (infrared processing) on nutrient and energy digestibilities in cereal grains. For this reason, a review is presented on the effect of different processing methods, with emphasis on micronization, on digestibility. Furthermore, studies were carried out with growing pigs on the digestibility of wheat shorts. Fiber is an important component in wheat shorts. A short review of fiber is therefore also provided, with particular reference to the digestion (fermentation) of fiber in the digestive tract of the pig.

A. Effect of Processing on Energy and Nutrient Digestibilities

Modification of cereal grains by processing and its effect on the nutritional quality for livestock and humans has been of interest for many years. Several researchers have shown different nutritional responses between processing methods such as extrusion, drum-drying, cooking, boiling, baking and micronization (Snow and O'Dea, 1981; Björck et al., 1984a,b; Hagander et al., 1985; Savage and Clark, 1988). Heat treatment is involved in all aforementioned processing methods, which may have both beneficial and undesirable effects on the nutritional value. Beneficial effects include destruction of antinutritional factors and gelatinization of starch. On the other hand, the Maillard reaction between protein and sugars reduces the nutritional value of protein.

Protein

Moderate heat processing has been reported to improve the digestibility of seed proteins by destroying protein inhibitors and other antinutritional substances and opening the protein structure through denaturation (Hsu et al., 1977). With increasing severity of heat treatment, protein digestibility decreases. The biological availability of amino acids may be affected through different mechanisms: the sulfur-containing amino acids are sensitive to oxidation and desulphurization; heating in the presence of reducing sugars leads to a decrease in the availability of lysine, particularly through the Maillard reaction (Hurrell and Carpenter, 1977).

It is well accepted that heat treatment inactivates antinutritive factors in raw soybean products, primarily the protein digestion inhibitors (Liener and Kakade, 1980). The degree of heat treatment has been shown to greatly affect the nutritional value of soybean products for swine (Rudolph et al., 1983). Excessive heat during processing can lead to destruction of amino acids and the formation of biologically unavailable amino acid-carbohydrate complexes (Mauron, 1981). The Maillard reaction involves the formation of complexes between reducing sugars and free amino groups in protein which leads to a decrease in both protein digestibility and amino acid availability. Dry heat treatment, as is applied in roasting or baking, is more damaging than autoclaving or pressure cooking in which water activities are close to 1 (Adrian, 1974). Maximum browning occurs at water activities between 0.3 and 0.7 depending on the type of food (Eichner, 1975). Lysine is the most reactive protein-bound amino acid due to its free ϵ-amino group. However, arginine, tryptophan, cysteine and histidine may also be affected (Hurrell and Carpenter, 1977). Because lysine is limiting in

cereal proteins, the fate of this amino acid during heat processing is particularly important. The range in heat treatments that effectively destroy antinutritional factors without damaging the nutritive value of protein has not been well defined.

The growth depression of animals fed high tannin sorghum is attributed to its inhibiting effect on the digestive enzymes and also to the formation of complexes with other proteins in the digestive tract, thereby rendering these resistant to enzymatic breakdown (McLeod, 1974). It has also been suggested that the formation of an insoluble tannin-protein complex in the hard outer corneous endosperm encapsulates the starch granules thereby protecting these from the action of digestive enzymes (Wall and Blessin, 1969). Consequently, any heat treatment which disrupts the tannin-protein complex should enhance the digestibility of grain as a whole.

Another example of the effect of processing on digestibility, in relation to heat application to inactivate antinutritional factors, was provided by Huisman et al. (1988). The ileal crude protein (CP) digestibilities in beans (*Phaseolus Vulgaris*) heated for 20, 40 and 60 minutes were -36.1, 8.3 and 37.3%, respectively. The increase in digestibility was accompanied by a decrease in the content of lectins. Amino acid digestibilities were not reported in these studies. The mode of action of lectins and effects on protein digestion and amino acid absorption were discussed in detail by Huisman (1989).

In addition to the aforementioned examples relating to heat treatment, fineness of grinding may affect CP and amino acid digestibility. Increasing the fineness of grinding improved the ileal amino acid digestibilities in wheat (Sauer et al., 1977) and sorghum (Owsley et al., 1981). Wüensche et al. (1988) showed a dramatic effect of fineness of

grinding on amino acid digestibility in barley and wheat. The apparent lysine digestibilities were 41, 57 and 67% in coarse, medium and finely ground barley, respectively. In the same order for wheat, these values were 70, 77 and 80%, respectively. These studies were carried out with the fecal analysis method. However, the ileal analysis method is expected to show, at least, similar relative responses. Several studies have shown that the ileal analysis method is more sensitive than the fecal method for determining differences in amino acid digestibility as these result from different processing methods (Sauer et al., 1977; Knabe et al., 1989).

Starch and Energy

Starch is the storage polysaccharide of higher plants and a major source of energy for animals. The content of starch in most cereal grains ranges from 60 to 80%. Starch is also a major component of many grain legumes. The livestock industry depends heavily on cereal grains as major sources of energy and protein. The structure and composition of cereal starches and their interactions with proteins play a major role in the digestibility and feeding value of grains.

Cooking and gelatinization of starch are known to increase the susceptibility to α-amylase hydrolysis, mainly due to hydration of starch granules and partial solubilization of starch molecules. However, according to McNeill et al. (1975), the solubility of the protein matrix encapsulating the starch granules is even more important than the degree of gelatinization to affect the digestibility of processed sorghum.

Gelatinization of wheat flour was studied by Chiang and Johnson (1977). An increase in the extrusion temperature resulted in a higher degree of gelatinization. In addition, an

increase in the moisture content of feed had a positive effect at high temperatures. Anderson (1968) compared roller, steam and extrusion cooking for gelatinization of corn grits. At comparable water absorption indexes suitable for production of food, extrusion cooking gave the highest water solubility index. Changes in carbohydrate solubility could affect ruminal carbohydrate digestion since nutrient solubility in rumen fluid is necessary for maximum utilization. However, the magnitude of the differences in carbohydrate profiles and solubilities were not large enough to explain the wide variation in the site of digestion of carbohydrate in the digestive tract (McNeill et al., 1975). Starch granules of micronized and steam-flaked grains were completely gelatinized and expanded many times their original size. Micronized starch was less susceptible to enzymatic hydrolysis even though it appeared to be gelatinized to a greater degree as indicated by a reduced viscosity peak height. Starch in steam-flaked grain was most susceptible to α-amylase, which indicated that the degree of gelatinization is not the only factor which affects the susceptibility of starch to the action of enzymes. The effect of processing method upon solubility of the protein matrix encapsulating starch granules in the endosperm seems to be the major factor affecting the efficiency of utilization. Therefore, processing methods which produce a change in the organization of the grain kernel to release starch granules from the protein matrix offer the promise of increasing carbohydrate utilization. Steam-flaking utilizes steam to soften the kernel prior to rolling, whereas micronization relies only upon kernel moisture to expand the kernel when it is heated with infrared radiation prior to rolling. Therefore, heat, water and pressure on the starch granules seem to be necessary for optimal gelatinization and structural disruption of the endosperm.

In addition to affecting the substrate, heat treatment also inactivates α-amylase inhibitors present in raw cereals. Processing may also affect the availability of carbohydrates by mechanically disrupting the structure of the material, thereby increasing the surface area to starch ratio (Björck et al., 1984b).

Micronization is a relatively new process that is increasingly used in the food and feed industry. Micronization is a short time high temperature process using water, heat and mechanical pressure to achieve conditions essential for optimum cooking and starch gelatinization. Micronization uses infrared wavelengths ranging from 1.8 to 3.4 microns. Infrared rays cannot be converted into heat until they strike absorbent material. This energy, when it penetrates the material, causes the constituent molecule to vibrate. The inter-molecular friction brings about rapid internal heating and a rise in water vapour pressure. For example, in cereal grains, internal temperatures of 90 °C are obtained in 50 seconds; in soybeans, internal temperatures of 110 to 115 °C are obtained in 90 seconds.

There is a scarcity of information on the effect of micronization on energy and amino acid digestibilities, as these are determined with the ileal analysis method, in cereal grains. Therefore, studies were carried out to determine the effect of micronization on nutrient and energy digestibilities in hulless barley and wheat fed to young pigs. The digestive system of the young pig is relatively immature and micronization of cereal grains may overcome this limitation.

B. Dietary Fiber and Digestion of Fiber in Pigs

A major problem in research on dietary fiber has been the development of suitable analytical methods. Dietary fiber was defined by Trowell et al. (1976) as the sum of plant polysaccharides and lignin that are resistant to breakdown by alimentary enzymes. Therefore, an enzymatic method was developed to remove starch and the residue defined chemically as non-starch polysaccharides (NSP) (Englyst, 1989). Dietary fiber has also been defined as neutral-detergent fiber (NDF) after extraction with a neutral-detergent solution (Van Soest and Wine, 1967) which consists of cellulose, hemicellulose and lignin, and acid-detergent fiber (ADF) after digestion with an acid-detergent solution (Van Soest, 1963) which consists of cellulose and lignin. The term dietary fiber is not synonymous with crude fiber. Crude fiber contains mainly cellulose and lignin because during the procedure for crude fiber determination hemicellulose and pectins are extracted.

Chemistry and properties of fiber components

Dietary fiber represents a heterogenous mixture of structural (cellulose, hemicellulose, and pectins) and non-structural polysaccharides (gums, mucilage, and algal types) and lignin (Low, 1985; Englyst, 1989; Potkins et al., 1991).

Cellulose is a long linear polymer of 1,4 β-linked glucose units. These straight chain polymers, packed side by side in plants, are stabilized by hydrogen bonds between hydroxyl groups of sugar residues forming strong intermolecular bonds, which makes it insoluble in water and provides structural strength. Cellulose is the only fiber component with a truly fibrous structure. Hemicellulose is a heterogenous group of substances containing a number of sugars in its backbone and side chains. The predominant sugars in hemicellulose are D-

xylose, D-mannose, D-galactose, L-arabinose, and 4-*O*-methyl-D-glucuronic acid. Hemicellulose, especially the hexose and uronic acid components, is more accessible to microbial enzymes than cellulose. Pectins are a complex group of polysaccharides in which galacturonic acid is the principal constituent. Other carbohydrate moieties may be linked to the galacturonic acid chain. Additional sugars sometimes found attached to the side chains include rhamnose, arabinose, xylose, and fucose. The backbone structure of pectin is an unbranched chain of 1,4-linked D-galacturonic acid units. These substances are usually water-soluble and gel forming. Lignin is the primary non-carbohydrate component of fiber and is a three-dimentional polymer composed of approximately 40 phenol units with strong intramolecular bonding. This strong intramolecular bonding makes lignin a very inert substance. Gums are hydrocolloids secreted at the site of plant injury by specialized secretory cells, which are composed of a variety of sugars and sugar derivatives. Gums consist primarily of glucuronic acid. Mucilages and algal polysaccharides are hydrocolloids and similar to gums in chemical structure.

Digestion of fiber in pigs

The digestibility of fiber components is affected by the source and the level of fiber in the diet. Stanogias and Pearce (1985) suggested that the extent of fiber digestibility depends primarily on the origin of fiber and to a lesser extent on the amount of fiber in the diet. Dietary fiber can be classified into two types. First, insoluble lignified fiber found in the secondary cell walls. Second, unlignified fiber found in the cell walls and middle lamellae of the endosperm, which is partially soluble. The first type of fiber is relatively resistant to microbial degradation in the gastrointestinal tract of monogastric animals and influences

transit time and fecal weight (Van Soest et al., 1983; Stephen and Cummings, 1980), whereas the second type is usually more susceptible to microbial fermentation, thus increasing bacterial activity in the large intestine (Stephen and Cummings, 1980). The fiber components from legume seeds were more digestible than those from cereal grains (Stanogias and Pearce, 1985), which also supports the premise that there is a relationship between the source of fiber (its chemical composition and physical properties) and its digestibility by the pig. Therefore, the digestibility of fiber itself is dependent on the relative amounts of cellulose, hemicellulose, pectins and lignin. Misleading information might be obtained when purified sources of fiber (for example Alphafloc) are used, because they differ considerably in properties from natural fiber sources (Van Soest, 1978). Purified sources may also be more resistant to bacterial fermentation in the large intestine of the pig.

There is a wide range in the digestibility of fiber from different sources. Forbes and Hamilton (1952) and Keys and DeBarthe (1974) fed pigs fiber from purified and natural sources and reported digestibilities of cellulose ranging from 21.0 to 92.1%. Baird et al. (1969) showed that pigs digested from 20.5 to 66.5% of crude fiber from different sources. Stanogias and Pearce (1985) reported that the digestibility values of NDF in different fiber sources ranged from 18.1 to 84.0%.

While the source of fiber is an important factor in determining the digestibility of fiber itself, there is controversy about the effect of level of fiber in the diet. Addition of purified cellulose to standard commercial pig diets resulted in a lower digestibility of crude fiber in high-fiber compared with low-fiber diets when these diets were fed ad libitum (Cunningham et al. 1962), at restricted levels (Farrell and Johnson, 1972) or at maintenance

levels (Gargallo and Zimmerman, 1980). When the intake of the diets used by Cunningham et al. (1962) was reduced to maintenance levels, the digestibilities of crude fiber in the high- and low-fiber diets were equal but higher ($P < .05$) than the digestibility of crude fiber in the same diets when these were fed ad libitum. These studies suggested that the effect of crude fiber *per sé* is more likely to be exerted through the actual amount of fiber intake rather than the proportion of fiber in the diet.

The apparent digestibilities of dry matter (DM), energy and nitrogen of the diets decreased linearly with increasing levels of NDF intake (Kennelly and Aherne, 1980a,b; Stanogias and Pearce, 1985). Apparently, increasing the level of fiber in the diet has a more depressive effect on the apparent digestibility of the non-fiber components in the diets than on the digestibility of the fiber components. The depressive effect of an increase in the intake of NDF on the apparent digestibility of DM, energy and nitrogen might be attributed to one or more of the following factors: a) faster rate of passage of food through the digestive tract (Gargallo and Zimmerman, 1981); b) increased excretion of metabolic and microbial nitrogen (Mason and Palmer, 1973); c) low availability of nitrogen and other nutrients in fiber (Forbes and Hamilton, 1952; Pals and Ewan, 1978); d) increased excretion of nitrogen and other nutrients bound or physically entrapped in the bulk of the bolus of fibrous digesta (Bailey et al., 1974; Eastwood and Kay, 1979).

The digestibility of fiber components in the small intestine of monogastric animals has become of interest in recent years. Dietary fiber is resistant to the action of digestive enzymes in the small intestine. However, a number of studies have shown that there is considerable microbial activity in the small intestine. Approximately 50% of hemicellulose

and cellulose and 90% of pectin disappeared in the small intestine (Rowan et al., 1992). Graham et al (1986) reported that up to 35% of NSP was degraded in the small intestine of pigs fed wheat bran or sugar beet pulp. Schulze et al. (1994) showed that the ileal digestibility of NDF purified from wheat bran ranged from 16 to 18%. Schulze et al. (1994) also showed that a considerable amount of CP (% N × 6.25) and amino acids are associated with the NDF fraction. However, there is no information in the literature on the ileal digestibility of CP and amino acids associated with NDF. As was mentioned previously, wheat shorts have a relatively high content of NDF and was therefore chosen as the feed ingredient to be investigated. Additional studies were carried out to determine the effect of fiber on the level of bacterial protein in ileal digesta and feces and mucin in ileal digesta.

C. Objective of Thesis

Studies were carried out to determine:

1. The effect of micronization on the digestibilities of energy, starch and amino acids in hulless barley and wheat fed to young pigs.
2. The variability in amino acid digestibility in growing pigs fed different samples of wheat shorts, which varied in NDF content. In addition, studies were carried out to determine the digestibilities of amino acids associated with NDF and the effect of NDF on the output of mucin and bacterial protein in digesta collected from the distal ileum and in feces.

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CHAPTER 2

EFFECT OF MICRONIZATION ON ENERGY, STARCH AND AMINO ACID DIGESTIBILITIES IN HULLESS BARLEY FOR YOUNG PIGS*

A. Introduction

Cereal grains serve as major sources of both energy and protein (amino acids) in diets for swine. The maturity of the digestive tract of growing and finishing pigs allows for efficient digestion of carbohydrates and protein in most cereals. However, there is a scarcity of information on the digestibility of carbohydrate and amino acids in young pigs, in which the digestive system is not yet fully developed (Bengala-Freire et al., 1988).

Commonly used cereal grains in diets for young pigs include wheat and corn. Limited amounts of hulled barley are sometimes also included. The development of hulless barley (HB) with a higher digestible energy content (compared to hulled barley) may result in increased usage of barley by young pigs.

There is no information on the effect of infrared processing (micronization) on energy and amino acid digestibilities in HB. As was pointed out previously (e.g., Sauer and Ozimek, 1986), the ileal rather than faecal analysis method should be used to determine amino acid digestibilities in feedstuffs for pigs, because of the modifying action of the microflora in the large intestine. On the other hand, the digestibility of energy should be determined with the faecal analysis method, as there can be considerable disappearance of energy in the large intestine in the form of volatile fatty acids (e.g., Fan et al., 1994).

The objectives of this study were to determine the effect of infrared processing on energy, starch and amino acid digestibilities in HB fed to young pigs.

B. Experimental Procedures

Animal Trial Procedures

Six PIC barrows (Canabrid x Camborough), weaned at 3 wk of age, were obtained from the University of Alberta Swine Research Unit. The average body weight (BW) at weaning was 7.1 kg. The barrows were housed individually in metabolic crates (height: 85 cm; length: 70 cm; width: 65 cm) in a barn in which the temperature was maintained between 25 and 28 °C. The pigs had *ad libitum* access to a starter diet containing 18% crude protein (CP) formulated to supply digestible energy and nutrients according to the National Research Council (NRC, 1988) recommendations. Water was freely available from a low-pressure drinking nipple.

The pigs were fitted with a simple T-cannula at the distal ileum, approximately 5 cm from the ileo-caecal sphincter, on d 6 or 7 after weaning. A detailed description of cannula preparation, surgery, pre-and post-operative care was previously provided by Li et al. (1994).

Following a 7-d recuperation period, the pigs were fed three experimental diets (Table 2-1) according to a repeated 3 × 3 Latin square design. Each experimental period was 9 d. The pigs were fed the experimental diets at a rate of 5% of the average BW of all pigs which was determined at the start of each experimental period. The average BW of the pigs were 9.3, 10.9 and 13.2 kg at the beginning of periods 1, 2 and 3, respectively. The BW at the conclusion of the experiment was 15.9 kg. The total daily allowances were offered in

three meals of equal amounts at 0800, 1600 and 2400 h, respectively. All pigs usually consumed their meal allowance within 1 h. The experimental diets consisted of hulless barley and soybean meal (HB + SBM), micronized hulless barley and soybean meal (MHB + SBM) and corn starch and soybean meal (C + SBM), (Table 2-1). The diets were fed in mash form and formulated to contain 18% CP (as-fed). The HB + SBM and MHB + SBM diets were formulated to contain 55% CP from soybean meal and 45% CP from HB. The variety of HB was CDC Buck (improved six row HB, released by the Crop Development Centre, University of Saskatchewan, SK). Soybean meal was solvent-extracted and contained 48.7% CP (as-fed). Canola oil was included in the diets to increase the digestible energy content to the level recommended by NRC (1988). Vitamins and minerals were also supplemented according to NRC (1988) standards. Chromic oxide was used as marker to determine the digestibilities of the parameters measured.

The collection of faeces was initiated at 0800 on day 6 of each experimental period and continued for 48 consecutive hours. Ileal digesta were collected for 24 h; from 0800 to 1600 h on day 8, from 2400 to 0800 h and from 1600 to 2400 h on day 9. The procedures for collection of faeces and digesta were previously described by Li et al. (1994). Faeces and digesta were frozen at - 28°C immediately following collection. Samples were pooled giving one sample of faeces and digesta for each pig in each period.

Hulless barley was processed at Infraready Products Limited (Saskatoon, SK) using a Micro Red 20 Cereal Micronizer (Micronizing Company Limited, Suffolk, UK). The barley was cleaned and then preconditioned with water to raise the moisture content to 18 to 20%. Thereafter, the HB was micronized for 50 sec. with infrared radiant energy (90 to 95 °C), and

passed through a roller mill.

The animals used in this experiment were cared for in accordance with the guidelines established by CCAC (1993) and approved by the Faculty of Agriculture, Forestry and Home Economics Animal Care Committee of the University of Alberta.

Chemical and Statistical Analysis

Samples of the diets were taken each time when the meal allowances were weighed out and pooled for each dietary treatment. Faeces and digesta were freeze-dried and finely ground.

Association of Official Analytical Chemists (AOAC) (1984) methods were used to determine dry matter (method no. 7.003). Gross energy was determined using a Parr 1241 Adiabatic Bomb Calorimeter (Parr Instruments, Moline, IL). Crude protein was determined with a Leco FP-428 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Analysis of neutral-detergent fiber (NDF) in the dietary ingredients were carried out according to principles outlined by Goering and Van Soest (1970). The contents of starch in the dietary ingredients, digesta and faeces were determined with the Megazyme total starch analysis procedure (thermostable α - amylase/amyloglucosidase method; Megazyme, Warriewood, Australia). Chromic oxide was measured according to Fenton and Fenton (1979).

For amino acid analyses, except for cysteine and methionine, approximately 0.1 g of sample was weighed into a screw-capped test tube and mixed with 3 ml of 6 N HCL. The tubes were purged with nitrogen and then hydrolyzed in an oven at 110 °C for 24 h. The hydrolyzed samples were mixed with the internal standard, DL-amino-n-butyric acid, and centrifuged at 1,110 × g for 15 min at 4°C. The supernatant of the sample was analyzed

according to principles outlined by Jones and Gilligan (1983) using a Varian 5000 high performance liquid chromatography system with a reverse-phase column and a Varian Fluorichrom detector (Varian Canada Inc., Mississauga, ON). This procedure was described in more detail by Sedgwick et al. (1991). The amino acids were derivatized with an o-phthaldialdehyde reagent solution. The mobile phase consisted of two solvents with a flow rate of 1.1 ml/min. Solvent A contained .1 M sodium acetate (pH 7.2), methanol, and tetrahydrofuran in a ratio of 90 to 5; solvent B was pure methanol. Peaks were recorded and integrated using the Ezchrom™ Chromatography Data System (version 2.12; Shimadzu Scientific Instruments Inc., Columbia, MD). Methionine and cysteine were determined as methionine sulfone and cysteic acid after oxidation with 98% performic acid overnight according to AOAC (1984; method no. 43.263). The oxidized samples were dried according to procedures described by Dugan et al. (1992), then hydrolyzed and analyzed in the same manner as the other amino acids. Tryptophan and proline were not measured.

Analyses of ingredients and diets were carried out in triplicate; analyses of faeces and digesta in duplicate. Dry matter, NDF, energy, starch, CP and amino acid contents of the ingredients and diets are presented in Tables 2-2 and 2-3, respectively.

The apparent ileal and faecal digestibilities of dry matter, energy, starch, CP and amino acids in the experimental diets were determined using equation (1):

$$D_D = 100\% - [(I_D \times A_F) / (A_D \times I_F)] \times 100\% \quad (1)$$

where D_D is apparent digestibility of a nutrient in the assay diet (%), I_D is marker concentration in the assay diet (%), A_F is nutrient concentration in ileal digesta and faeces (%), A_D is nutrient concentration in the assay diet (%), I_F is marker concentration in ileal

digesta or faeces (%)

By using soybean meal as the basal feed ingredient, the apparent ileal digestibility values of CP and amino acids in HB and MHB were calculated by the difference method using equation (2):

$$D_A = [\, D_D - (D_B \times S_B)] \,/\, S_A \qquad (2)$$

where D_A is apparent digestibility of a nutrient in the assay feed ingredient (%), D_D is as defined previously, D_B is apparent digestibility of a nutrient in the basal feed ingredient (%), S_B is the contribution level (%) of a nutrient in the basal ingredient to the assay diet, S_A is the contribution level (%) of a nutrient in the assay ingredient to the assay diet.

Data were subjected to statistical analysis using the General Linear Model Procedure of SAS (1988). Means of treatments were compared using the Student-Newman-Keuls multiple range test procedure.

C. Results and Discussion

The piglets remained healthy and consumed their meal allowances throughout the experiment. Postmortem examinations, conducted at the conclusion of the experiment, revealed no adhesions or other intestinal abnormalities.

The chemical composition of HB and MHB and the experimental diets are presented in Tables 2-2 and 2-3, respectively. Micronization of HB resulted in a small decrease in its starch content, from 63.9 to 60.2% which is in agreement with McNeill et al. (1975) in studies with sorghum. These authors reported that micronization decreased the contents of total carbohydrates and starch; there was an increase in the ethanol-soluble carbohydrate

fraction. Douglas et al. (1991) in studies with corn and sorghum also reported that micronization caused only minor changes in the chemical composition, including amino acid content. Furthermore, the analyzed values of CP and amino acids in the experimental diets were very close to the calculated values based on the analyzed values in HB, MHB and SBM.

The apparent ileal digestibilities of the parameters measured are presented in Table 2-4. The digestibilities of dry matter, energy, starch and CP were higher ($P < 0.05$) in the MHB + SBM than HB + SBM diet. The differences were 10.5, 18.3 and 5.2 percentage units for energy, starch and CP, respectively. The digestibilities of most of the amino acids were also higher ($P < 0.05$) in the MHB + SBM than HB + SBM diet. Of the indispensable amino acids, except for lysine, the differences were significant ($P < 0.05$), ranging from 3.2 (arginine) to 5.3 (threonine) percentage units; of the dispensable amino acids, except for aspartic acid and cysteine, the differences were significant ($P < 0.05$) ranging from 2.1 (aspartic acid) to 9.9 (glycine) percentage units. As was expected, the digestibilities of amino acids in the C + SBM diet were usually higher than in the HB + SBM or MHB + SBM diets.

The apparent faecal digestibilities of the parameters measured are presented in Table 2-5. As was the case for the ileal digestibilities, the faecal digestibilities for dry matter, energy, starch, CP and amino acids were higher in the MHB + SBM than HB + SBM diet. The differences were 2.0, 2.6, 0.3 and 6.2 percentage units for dry matter, energy, starch and CP, respectively. For the indispensable amino acids, the differences ranged from 2.8 (arginine) to 10.2 (methionine) percentage units; for the dispensable amino acids, the differences ranged from 1.6 (glutamic acid) to 7.3 (glycine) percentage units.

There are no reports in the literature on the effect of micronization on energy and nutrient digestibilities in diets containing barley, including HB, in pigs or other monogastric species. Micronization is a process using infrared generators to heat the grain with or without pre-conditioning with water. In these studies, the grain was heated to 90 °C in 50 sec. and pre-conditioned with water to raise its moisture content to 18-20%. This process will result in gelatinization of starch. The protein matrix of the kernel is disrupted and many of the starch granules are ruptured and adhere together forming sheets (Harbers, 1975). On the other hand, several studies have been carried out to determine the effect of micronization on the nutritive value of sorghum (McNeill et al., 1975; Savage et al., 1980; Savage and Clark, 1988; Douglas et al., 1991). Savage et al. (1980) reported that micronization increased in vitro starch availability of sorghum and improved apparent digestibility of dry matter. McNeill et al. (1975) reported that starch in micronized sorghum was more susceptible to enzymatic degradation by amyloglucosidase than in untreated sorghum. Douglas et al. (1991) reported that micronization improved in vitro starch digestibility in both sorghum and corn and performance of broilers fed diets containing these cereals.

There is a scarcity of information on the effect of micronization on CP and none on amino acid digestibility. However, results from other studies in which different processing methods involving heat were used can be referred to. For example, Rooney et al. (1986), in studies with sorghum, suggested that cooking increased the solubility of protein in general and that of the prolamin proteins in particular. Hsu et al. (1977) reported that heating improved the digestibility of seed protein by destroying protein inhibitors and opening the structure through denaturation. Baking increased the ileal digestibility of CP (and also of

starch and dietary fiber) of a HB-based diet fed to pigs (Fadel et al., 1989). Microwave heating of soybean for 9, 12 and 15 min increased the true protein digestibility in rats from 73 to 84, 87 and 81%, respectively (Hafez et al., 1985)

The effect of micronization on the disappearance of the parameters measured in the large intestine, expressed quantitatively as g kg^{-1} DMI, is presented in Table 2-6. The disappearance of dry matter, energy and starch in the large intestine was lower ($P < 0.05$) in pigs fed the MHB + SBM than the HB + SBM diet. Micronization, therefore, resulted in an increase in the digestion and absorption of energy in the small intestine and a decrease of microbial fermentation of energy in the large intestine. This shift in the disappearance of energy from the large to the small intestine should also result in an improvement in the efficiency of energy utilization, as was shown by Just et al. (1983) in studies with growing pigs fed diets differing in fiber content. Irrespective of whether the MHB + SBM or HB + SBM diet was fed, starch escaping digestion in the small intestine was nearly completely fermented by the microflora in the large intestine (Tables 2-4 and 2-5), which was also observed in studies by Sauer et al. (1977) and Fadel et al. (1989). Furthermore, for each of the experimental diets, there was a larger disappearance from the large intestine of the dispensable than indispensable amino acids. Of the dispensable amino acids, the disappearance was largest for aspartic acid, glutamic acid and glycine. The reasons for the relatively large disappearance of these amino acids in the large intestine was previously discussed by Li et al. (1994). There was net synthesis of methionine in the large intestine of pigs fed the HB + SBM and MHB + SBM diets. Net synthesis of methionine was also reported in other studies (e.g., Li and Sauer, 1994).

The apparent ileal amino acid digestibilities in HB and MHB (Table 2-7) were determined with the difference method. As was shown by Fan and Sauer (1995), amino acid digestibilities in ingredients low in amino acid content (e.g., barley) should be determined with the difference rather than direct method as the direct method will underestimate the digestibility values. In the studies by Fan and Sauer (1995), of the indispensable amino acids, the underestimations ranged from 2.4 (histidine) to 9.1 (threonine) percentage units. With respect to the direct method, as was pointed out by Fan et al. (1994), the apparent amino acid digestibility values of amino acids in a feedstuff are dependent on their respective dietary amino acid levels. The apparent digestibilities of the indispensable amino acids were higher in MHB than HB and ranged from 5.3 (valine) to 10.0 (methionine) percentage units. Of the indispensable amino acids, the differences were significant at 5% for arginine, histidine, isoleucine, leucine, phenylalanine and valine and at 10% for threonine. The differences in digestibilities of the dispensable amino acids between MHB and HB ranged from 1.0 (aspartic acid) to 15.2 (glycine) percentage units. Micronization of HB improved ($P < 0.05$) the ileal digestibility of starch from 79.0 to 97.3%. The ileal digestibilities of starch in the HB + SBM and MHB + SBM diets (Table 2-4) were considered to be similar to HB and MHB, respectively, since SBM contributes only a negligible amount of starch (0.8%) to the total dietary starch content.

This is the first time that ileal amino acid digestibilities in HB have been reported in the literature. It is rather difficult to compare these values with those in the literature for hulled barley. First of all, there is considerable variation in amino acid digestibility values in hulled barley which is dependent on several factors, some of which were discussed by

Sauer and Ozimek (1986). Secondly, as was discussed previously, digestibility values are affected by method of determination. In most studies, digestibility values were determined with the direct method. Thirdly, BW (age) in young pigs may affect digestibility values. The digestibility values in studies with young pigs fed corn starch-based soybean meal diets were lower during the first than second experimental period (Li et al., 1993; Li and Sauer, 1994). Fan and Sauer (1995), in studies with older pigs (BW: 40 to 70 kg) in which the difference method was used also, reported ileal lysine and threonine digestibilities of 61.3 and 62.4% in hulled barley, respectively, which compare to 56.1 and 55.6% respectively with young pigs (BW: 7.1 to 15.9 kg) in the present studies.

In conclusion, micronization of HB improved the digestibility of energy and the ileal digestibilities of most amino acids. Micronization also shifted the disappearance of starch from the large to the small intestine which may result in an improvement in the efficiency of energy utilization.

Table 2-1. Formulation (%) of the experimental diets

Ingredients	Diets[z]		
	HB + SBM	MHB + SBM	C + SBM
Hulless barley	72.34	-	-
Micronized hulless barley	-	72.32	-
Soybean meal	20.42	20.42	37.12
Corn starch[y]	-	-	55.31
Canola oil	3.00	3.00	3.00
Biophos[x]	1.28	1.30	1.95
Calcium carbonate	1.26	1.26	0.92
Trace-mineralized salt[w]	0.30	0.30	0.30
Vitamin-mineral premix[v]	1.00	1.00	1.00
Antibiotics[u]	0.10	0.10	0.10
Chromic oxide	0.30	0.30	0.30

[z] HB + SBM: hulless barley-soybean meal diet; MHB + SBM: micronized hulless barley-soybean meal diet; C + SBM: corn starch-soybean meal diet.

[y] St. Lawrence Starch Company Ltd., Mississauga, ON.

[x] Provided (%): available phosphorous, 15 - 18 and calcium, 24. Supplied by Continental Lime Ltd., Exshaw, AB.

[w] Windsor Salt Co., Toronto, ON. Composition (%): NaCl, 96.5; ZnO, .4; $FeCO_3$, .16; MnO, .12; CuO, .033; $Ca(IO_3)_2$, .007; CaO, .004.

[v] Provided the following (per kg diet): vitamin A, 10,000 IU; vitamin D_3, 1,000 IU; vitamin E, 80 IU; vitamin K_3, 2.0 mg; vitamin B_{12}, .03 mg; riboflavin, 12 mg; niacin, 40 mg; d-pantothenic acid, 25 mg ; choline, 1,000 mg; d-biotin, 025 mg; folic acid, 1.6 mg; thiamine, 3.0 mg; Ethoxyquin, 25 mg; pyridoxine, 2.25 mg. Fe, 150 mg; Zn, 150 mg; Cu, 125 mg; I, .21 mg; Se, .3 mg. Supplied by Hoffmann-LaRoche Ltd., 2455 Meadowpine Blvd., Mississauga, ON.

[u] Veterinary LS-20 premix, provided (g/kg mixture): Lincomycin hydrochloride 22, Spectinomycin sulphate 22. Supplied by the Upjohn Company, Animal Health Division, Orangeville, ON.

Table 2-2. Dry matter, neutral-detergent fiber, energy, starch, crude protein and amino acid content (%)[z] of the dietary ingredients

Item	Ingredients[y]		
	HB	MHB	SBM
Dry matter	87.3	88.6	88.7
Neutral-detergent fiber	11.0	10.6	7.1
Energy(MJ kg^{-1})	18.7	18.7	19.7
Starch	63.9	60.2	1.7
Crude protein	14.2	14.3	55.0
Amino acids			
Indispensable			
Arginine	0.52	0.54	3.58
Histidine	0.24	0.24	1.35
Isoleucine	0.43	0.46	2.41
Leucine	0.81	0.86	3.92
Lysine	0.38	0.37	3.25
Methionine	0.23	0.24	0.68
Phenylalanine	0.62	0.64	2.55
Threonine	0.32	0.35	1.82
Valine	0.60	0.63	2.49
Dispensable			
Alanine	0.45	0.49	2.53
Aspartic acid	0.65	0.69	5.76
Cysteine	0.25	0.25	0.54
Glutamic acid	3.09	3.25	9.76
Glycine	0.41	0.41	1.91
Serine	0.48	0.50	2.51
Tyrosine	0.31	0.34	1.72

[z] Dry matter basis

[y] Refer to Table 2-1.

Table 2-3. Dry matter, neutral-detergent fiber, energy, starch, crude protein and amino acid content (%)[z] of the experimental diets

Item	Diets[y]		
	HB + SBM	MHB + SBM	C + SBM
Dry matter	89.2	89.9	90.7
Neutral-detergent fiber	9.2	9.0	2.6
Energy (MJ kg^{-1})	19.1	19.1	18.5
Starch	45.5	43.2	58.9
Crude protein	21.1	21.1	19.8
Amino acids			
Indispensable			
Arginine	1.09	1.11	1.30
Histidine	0.45	0.45	0.50
Isoleucine	0.80	0.81	0.88
Leucine	1.38	1.40	1.42
Lysine	0.93	0.92	1.18
Methionine	0.30	0.30	0.23
Phenylalanine	0.95	0.97	0.93
Threonine	0.59	0.61	0.66
Valine	0.93	0.95	0.63
Dispensable			
Alanine	0.83	0.86	0.91
Aspartic acid	1.64	1.65	2.09
Cysteine	0.33	0.32	0.18
Glutamic acid	4.17	4.28	3.54
Glycine	0.67	0.68	0.69
Serine	0.85	0.86	0.91
Tyrosine	0.57	0.59	0.63

[z] Dry matter basis.

[y] Refer to Table 2-1.

Table 2-4. Apparent ileal digestibilities (%) of dry matter, energy, starch, crude protein and amino acids in the experimental diets

Item	Diets[z]			SEM[y]
	HB + SBM	MHB + SBM	C + SBM	
Dry matter	56.7[c]	67.4[b]	76.0[a]	1.29
Energy	59.3[c]	69.8[b]	79.5[a]	1.25
Starch	79.0[b]	97.3[a]	97.9[a]	0.65
Crude protein	68.0[c]	73.2[b]	76.9[a]	1.09
Amino acids				
Indispensable				
Arginine	84.2[c]	87.4[b]	91.5[a]	0.57
Histidine	83.3[c]	87.9[b]	91.2[a]	0.59
Isoleucine	79.2[c]	83.4[b]	86.5[a]	0.82
Leucine	79.3[b]	83.7[a]	85.6[a]	0.76
Lysine	78.4[b]	81.7[b]	87.9[a]	1.02
Methionine	70.2[c]	75.9[b]	81.4[a]	1.10
Phenylalanine	79.5[c]	84.4[b]	86.3[a]	0.70
Threonine	69.7[b]	75.0[a]	77.4[a]	1.40
Valine	77.4[b]	82.2[a]	83.9[a]	0.86
Dispensable				
Alanine	73.2[c]	78.0[b]	83.6[a]	1.04
Aspartic acid	78.4[b]	80.5[b]	85.9[a]	0.95
Cysteine	63.5	68.8	64.1	1.79
Glutamic acid	81.0[b]	87.9[a]	89.6[a]	0.77
Glycine	60.4[b]	70.3[a]	72.5[a]	2.74
Serine	77.2[c]	81.2[b]	84.7[a]	0.86
Tyrosine	78.6[c]	81.9[b]	88.1[a]	0.80

[z] Refer to Table 2-1.

[y] Standard error of the mean.

[a-c] Means in the same row with different superscript letters differ ($P < .05$).

Table 2-5. Apparent faecal digestibilities (%) of dry matter, energy, starch, crude protein and amino acids in the experimental diets

Item	Diets[z]			SEM[y]
	HB + SBM	MHB + SBM	C + SBM	
Dry matter	85.4[c]	87.4[b]	92.9[a]	0.24
Energy	84.8[c]	87.4[b]	94.1[a]	0.29
Starch	99.6[b]	99.9[a]	100.0[a]	0.05
Crude protein	76.8[c]	83.0[b]	90.2[a]	0.71
Amino acids				
Indispensable				
Arginine	89.3[c]	92.1[b]	96.8[a]	0.41
Histidine	85.2[c]	89.1[b]	95.3[a]	0.56
Isoleucine	79.2[c]	85.1[b]	92.4[a]	0.71
Leucine	84.1[b]	88.1[a]	93.6[a]	0.49
Lysine	81.2[b]	85.8[b]	94.5[a]	0.87
Methionine	63.2[c]	73.4[b]	82.9[a]	1.64
Phenylalanine	83.9[c]	88.5[b]	93.3[a]	0.50
Threonine	75.1[c]	81.9[b]	90.3[a]	0.88
Valine	79.3[b]	85.4[a]	91.9[a]	0.65
Dispensable				
Alanine	80.5[c]	85.0[b]	92.0[a]	0.52
Aspartic acid	85.7[c]	88.7[b]	95.0[a]	0.40
Cysteine	79.5[c]	84.2[b]	87.2[a]	0.92
Glutamic acid	93.6[c]	95.2[b]	97.0[a]	0.17
Glycine	74.7[c]	82.0[b]	89.7[a]	0.96
Serine	82.1[b]	85.7[b]	94.3[a]	2.09
Tyrosine	83.3[c]	88.0[b]	93.8[a]	0.94

[z] Refer to Table 2-1.

[y] Standard error of the mean

[a-c] Means in the same row with different superscript letters differ ($P < .05$)

Table 2-6. Disappearance[z] of dry matter, energy, starch, crude protein and amino acids in the large intestine of pigs fed the experimental diets

Item	Diets[y]			SEM[x]
	HB + SBM	MHB + SBM	C + SBM	
Dry matter	286.4[a]	200.4[b]	169.3[b]	11.95
Energy (MJ kg^{-1})	4.9[a]	3.4[b]	2.8[c]	0.57
Starch	94.2[a]	11.3[b]	6.4[b]	3.31
Crude protein	18.7[b]	20.7[b]	25.7[a]	1.43
Amino acids				
Indispensable				
Arginine	0.56	0.52	0.69	0.07
Histidine	0.09[b]	0.05[c]	0.21[a]	0.02
Isoleucine	0.00	0.14	0.52	0.08
Leucine	0.66[b]	0.62[b]	1.14[a]	0.11
Lysine	0.26	0.38	0.78	0.11
Methionine	-0.21[c]	-0.08[b]	0.03[a]	0.03
Phenylalanine	0.42[b]	0.40[b]	0.65[a]	0.07
Threonine	0.32	0.42	0.85	0.08
Valine	0.18[b]	0.30[a]	0.50[a]	0.09
Dispensable				
Alanine	0.61	0.60	0.76	0.09
Aspartic acid	1.20[b]	1.35[b]	1.91[a]	0.16
Cysteine	0.53	0.49	0.42	0.04
Glutamic acid	5.25[a]	3.12[b]	2.62[c]	0.29
Glycine	0.96	0.80	1.19	0.19
Serine	0.42[b]	0.39[b]	0.87[a]	0.20
Tyrosine	0.27[b]	0.36[a]	0.36[a]	0.04

[z] g/kg DMI

[y] Refer to Table 2-1.

[x] Standard error of the mean.

[a-c] Means in the same row with different superscript letters differ ($P < .05$).

Table 2-7. Effect of micronization of hulless barley on the apparent ileal digestibilities (%) of starch, crude protein and amino acids

Item	HB[z]	MHB[z]	SEM[y]
Starch	79.0[a]	97.3[b]	0.49
Crude protein	60.8	69.2	2.05
Amino acids			
Indispensable			
Arginine	70.3[a]	79.6[b]	0.99
Histidine	75.2[a]	82.7[b]	0.84
Isoleucine	69.0[a]	76.1[b]	1.37
Leucine	73.7[a]	80.4[b]	1.42
Lysine	56.1	63.5	3.18
Methionine	63.3[a]	73.3[b]	1.80
Phenylalanine	72.5[a]	80.0[b]	1.19
Threonine	55.6	65.0	3.17
Valine	72.0[a]	77.3[b]	1.43
Dispensable			
Alanine	60.0	68.7	2.24
Aspartic acid	64.5	65.5	2.55
Cysteine	65.9	70.8	3.92
Glutamic acid	75.8[a]	86.1[b]	0.92
Glycine	47.6	62.8	4.36
Serine	70.8[a]	75.1[b]	1.75
Tyrosine	66.4[a]	78.1[b]	1.63

[z] Refer to Table 2-1.

[y] Standard error of the mean.

[a,b] Means in the same row with different superscript letters differ ($P < .05$).

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CHAPTER 3

EFFECT OF MICRONIZATION ON ENERGY, STARCH AND AMINO ACID DIGESTIBILITIES IN WHEAT FOR YOUNG PIGS

A. Introduction

Wheat is often included in diets for young pigs and serves as a major source of energy and to a lesser extent of protein. The maturity of the digestive tract of growing and finishing pigs allows for efficient digestion of carbohydrates and protein in wheat. However, there is little information on the digestibilities in young pigs, in which the digestive system is not yet fully developed (Bengala-Freire et al., 1988).

Several studies, in vitro or in vivo using rats, have been carried out to determine the effect of processing methods such as extrusion, drum-drying, extrusion and cooking on the nutritive value of wheat (Björck and Asp, 1984; Björck et al., 1984a, b; Jideani et al., 1994). Depending on the severity of processing conditions, processing may have a beneficial or detrimental effect (Björck and Asp, 1983). Beneficial effects include gelatinization of starch. Detrimental effects include Maillard reactions between protein and sugars.

The objectives of this study were to determine the effect of micronization (infrared processing) on the digestibilities of energy, starch, and amino acids in wheat fed to young pigs. The pigs were fitted with a simple T-cannula at the distal ileum which allowed for measurements of the disappearance of energy and starch in the small intestine and ileal amino acid digestibilities.

B. Experimental Procedures

Animal Trial Procedures

Six PIC (Pig Improvement Canada) barrows (Canabrid x Camborough), weaned at 3 wk of age, were obtained from the University of Alberta Swine Research Unit. The average BW at weaning was 7.0 kg. The barrows were housed individually in metabolism crates (height: 85 cm; length: 70 cm; width: 65 cm) in a barn in which the temperature was maintained between 25 and 28 °C. The pigs had *ad libitum* access to a starter diet containing 18% crude protein (CP) formulated to supply digestible energy and nutrients according to NRC (1988) recommendations. Water was freely available from a low-pressure drinking nipple.

The pigs were fitted with a simple T-cannula at the distal ileum, approximately 5 cm from the ileo-caecal sphincter, on d 6 or 7 after weaning. A detailed description of cannula preparation, surgery, pre-and post-operative care was previously provided by Li et al. (1994).

Following a 7-d recuperation period, the pigs were fed three experimental diets (Table 3-1) according to repeated Latin square design. Each experimental period was 9 d. The pigs were fed the experimental diets at a rate of 5% of the average body weight (BW) of all pigs which was determined at the start of each experimental period. The average BW of the pigs were 9.2, 11.0 and 13.4 kg at the beginning of periods 1, 2 and 3, respectively. The BW at the conclusion of the experiment was 16.5 kg. The total daily allowances were offered in three meals of equal amounts at 0800, 1600 and 2400 h, respectively. All pigs usually consumed their meal allowance within 1 h. The experimental diets consisted of ground wheat and soybean meal (W + SBM), micronized wheat and soybean meal (MW +

SBM) and corn starch and soybean meal (C + SBM) (Table 3-1). The diets were fed in mash form and formulated to contain 18% CP (as-fed). The W + SBM and MW + SBM diets were formulated to contain 55% CP from soybean meal and 45% CP from wheat. Soybean meal was solvent-extracted and contained 48.7% CP (as-fed). Canola oil was included in the diets to increase the digestible energy content to the level recommended by NRC (1988). Vitamins and minerals were also supplemented according to NRC (1988) standards. Chromic oxide was used as marker to determine the digestibilities of the parameters measured.

The wheat used in this study was Canada Prairie Spring Red which is a classification of wheat that usually refers to common feed wheats. Wheat used in the W + SBM diet was ground through a 3-mm mesh screen prior to diet formulation. Wheat used in the MW + SBM diet was processed at Infraready Products Limited (Saskatoon, SK, Canada) using a Micro Red 20 Cereal Micronizer (Micronizing Company Limited, Suffolk, UK). The wheat was cleaned and then preconditioned with water to raise its moisture content to 18 to 20%. Thereafter, the wheat was micronized for 50 sec. with infrared radiant energy (90 to 95 °C), and passed through a roller mill.

The collection of faeces was initiated at 0800 on day 6 of each experimental period and continued for 48 consecutive hours. Ileal digesta were collected for 24 h; from 0800 to 1600 h on day 8, from 2400 to 0800 h and from 1600 to 2400 h on day 9. The procedures for collection of faeces and digesta were previously described by Li et al. (1994). Faeces and digesta were frozen at -28°C immediately following collection. Samples were pooled giving one sample of faeces and digesta for each pig in each period.

The animals used in this experiment were cared for in accordance with the guidelines

established by CCAC (1993) and approved by the Faculty of Agriculture, Forestry and Home Economics Animal Care Committee of the University of Alberta.

Chemical and Statistical Analysis

Samples of the diets were taken each time when the meal allowances were weighed out and pooled for each dietary treatment. Faeces and digesta were freeze-dried and finely ground.

The dry matter content was determined according to AOAC (1990). Gross energy was determined using a Parr 1241 Adiabatic Bomb Calorimeter (Parr Instruments, Moline, IL, U.S.A.). Crude protein was determined with a Leco FP-428 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI, U.S.A.). Analysis of neutral-detergent fiber (NDF) in the dietary ingredients were carried out according to principles outlined by Goering and van Soest (1970). The contents of starch in the dietary ingredients, digesta and faeces were determined with the Megazyme total starch analysis procedure (thermostable α-amylase/amyloglucosidase method; Megazyme, Warriewood, Australia). Chromic oxide was measured according to Fenton and Fenton (1979).

For amino acid analyses, except for cysteine and methionine, approximately .1 g of sample was weighed into a screw-capped test tube and mixed with 3 ml of 6 N HCL. The tubes were purged with nitrogen and then hydrolyzed in an oven at 110 °C for 24 h. The hydrolyzed samples were mixed with the internal standard, DL-amino-n-butyric acid, and centrifuged at 1,110 × g for 15 min at 4°C. The supernatant of the sample was analyzed according to principles outlined by Jones and Gilligan (1983) using a Varian 5000 high performance liquid chromatography system with a reverse-phase column and a Varian

Fluorichrom detector (Varian Canada Inc., Mississauga, ON, Canada). This procedure was described in more detail by Sedgwick et al. (1991). The amino acids were derivatized with an o-phthaldialdehyde reagent solution. The mobile phase consisted of two solvents with a flow rate of 1.1 ml/min. Solvent A contained .1 M sodium acetate (pH 7.2), methanol, and tetrahydrofuran in a ratio of 90 to 5; solvent B was pure methanol. Peaks were recorded and integrated using the Ezchrom™ Chromatography Data System (version 2.12; Shimadzu Scientific Instruments Inc., Columbia, MD, U.S.A.). Methionine and cysteine were determined as methionine sulfone and cysteic acid after oxidation with 98% performic acid overnight according to AOAC (1990). The oxidized samples were dried according to procedures described by Dugan et al. (1992), then hydrolyzed and analyzed in the same manner as the other amino acids. Tryptophan and proline were not measured.

Analyses of ingredients and diets were carried out in triplicate; analyses of faeces and digesta in duplicate. Dry matter, NDF, energy, starch, CP and amino acid contents of the ingredients and diets are presented in Tables 3-2 and 3-3, respectively.

The apparent ileal and faecal digestibilities of dry matter, energy, starch, CP and amino acids in the experimental diets were determined using equation (1):

$$D_D = 100\% - [(I_D \times A_F) / (A_D \times I_F)] \times 100\% \qquad (1)$$

where D_D is apparent digestibility of a nutrient in the assay diet (%), I_D is marker concentration in the assay diet (%), A_F is nutrient concentration in ileal digesta and faeces (%), A_D is nutrient concentration in the assay diet (%), I_F is marker concentration in ileal digesta or faeces (%)

By using soybean meal as the basal feed ingredient, the apparent ileal digestibility

values of CP and amino acids in HB and MHB were calculated by the difference method using equation (2):

$$D_A = [D_D - (D_B \times S_B)] / S_A \quad (2)$$

where D_A is apparent digestibility of a nutrient in the assay feed ingredient (%), D_D is as defined previously, D_B is apparent digestibility of a nutrient in the basal feed ingredient (%), S_B is the contribution level (%) of a nutrient in the basal ingredient to the assay diet, S_A is the contribution level (%) of a nutrient in the assay ingredient to the assay diet.

Data were subjected to analysis of variance using the General Linear Model procedure of SAS (1988). Means of treatments were compared using the Student-Newman-Keuls multiple range test procedure.

C. Results and Discussion

The piglets remained healthy and consumed their meal allowances throughout the experiment. Postmortem examinations, conducted at the conclusion of the experiment, revealed no adhesions or other intestinal abnormalities.

Micronization is a process in which infrared generators are used to heat cereal grains with or without pre-conditioning with water. In this study, wheat was pre-conditioned with water to raise its moisture content to 18 to 20 % and then heated to 90 °C in 50 sec. This process will cause starch gelatinization, in which many of the starch granules are disrupted and adhere together forming sheets (Harbers, 1975).

The chemical composition of ground and micronized wheat and the experimental diets are presented in Tables 3-2 and 3-3, respectively. The analyzed values of CP and amino

acids in the experimental diets were very close to the calculated values based on the analyzed values in ground wheat, micronized wheat and soybean meal. Infrared processing resulted in a slight decrease, from 76.9 to 74.4%, in the content of starch in wheat (Table 3-2). McNeill et al. (1975) also reported a decrease in starch content, with a concomitant increase in the fraction of ethanol-soluble carbohydrates. In agreement with studies by Douglas et al. (1991) with corn and sorghum, micronization did not affect the content of the other parameters measured, including amino acids.

The apparent ileal digestibility values are presented in Table 3-4. The digestibilities of dry matter, energy and starch were higher ($P < .05$) in the MW + SBM than W + SBM diet. The differences were 10.7, 10.7 and 6.4 percentage units, respectively. The digestibilities of amino acids were higher in the MW + SBM than W + SBM diet. Of the indispensable amino acids, except for methionine, the differences were significant ($P < .05$), ranging from 2.5 (arginine) to 9.0 (threonine) percentage units. Of the dispensable amino acids, except for cysteine and glycine, the differences were significant ($P < .05$) ranging from 4.0 (aspartic acid) to 6.3 (alanine) percentage units.

As was reported in previous studies, the ileal compared with the faecal analysis method was more sensitive for detecting differences in amino acid digestibilities as these result from different processing methods (e.g., Sauer et al., 1977; Van Weerden et al.,1985). These studies show once more the modifying and apparent equalizing effect of the microflora in the large intestine on amino acid digestibility values.

With the exception of methionine and cysteine, the ileal digestibilities of amino acids were higher ($P < .05$) in the MW + SBM than W + SBM diet (Table 3-4). With the exception

of one study, there is no information in the literature on the effect of micronization on protein digestibility. There is no information on amino acid digestibility. Hafez et al. (1985) showed that microwave heating of soybean for 9 min increased the true protein digestibility in rats from 73 to 84%. Furthermore, the results of the present study are in agreement with studies in which other heat-processing methods were used. Hsu et al. (1977) reported that heating improved the digestibility of seed protein by destroying protein digestion inhibitors and opening the structure through denaturation. Rooney et al. (1986), in studies with sorghum, suggested that cooking increased the solubility of protein in general and that of the prolamin proteins in particular. Furthermore, baking increased the ileal digestibility of CP (also of starch and dietary fibre) of a hulless barley-based diet fed to pigs (Fadel et al., 1989).

The apparent faecal digestibility values are presented in Table 3-5. As was the case for the ileal digestibilities, the faecal digestibilities for dry matter, energy, CP and amino acids were higher in the MW + SBM than W + SBM diet. The differences were 1.1, 1.6 and 1.7 percentage units for dry matter, energy and CP, respectively. The faecal digestibility of starch in both diets was 100%. For the indispensable amino acids, the differences ranged from .5 (histidine) to 4.1 (methionine) percentage units; for the dispensable amino acids, the differences ranged from .2 (glutamic acid) to 2.4 (tyrosine) percentage units.

The effect of micronization on the disappearance of the parameters measured in the large intestine, expressed quantitatively as g kg^{-1} DMI, is presented in Table 3-6. The disappearance of dry matter, energy and starch in the large intestine was lower ($P < .05$) in pigs fed the MW + SBM than the W + SBM diet. For each of the experimental diets, there was a larger disappearance of the dispensable than indispensable amino acids. Of the

dispensable amino acids, the disappearance was largest for aspartic acid, glutamic acid and glycine. There was net synthesis of methionine in the large intestine of pigs fed the W + SBM diet.

The ileal and faecal digestibilities of energy as well as the ileal digestibility of starch were higher in the MW + SBM than W + SBM diet (Tables 3-4 and 3-5) which supports the results of studies in which micronization of sorghum and corn improved the in vitro availability of starch (Savage et al., 1980; Douglas et al., 1991). Starch granules of micronized sorghum were completely gelatinized and more susceptible to enzymatic degradation by α-amylase than starch of untreated ground sorghum (McNeill et al., 1975). Harbers (1975) showed that micronization disrupted the protein matrix surrounding starch granules thereby rendering starch more susceptible to enzymatic action.

The disappearance of dry matter, energy and starch in the large intestine was lower ($P < .05$) in pigs fed the MW + SBM than W + SBM diet (Table 3-6). Micronization shifted the disappearance of energy from the large to the small intestine. This shift should result in an improvement in the efficiency of energy utilization as was shown by Just et al. (1983) in studies with pigs fed diets with different fibre content. A one percent increase in the proportion of energy disappearing in the large intestine decreased the utilization of metabolizable energy by .8% (Just et al., 1983). According to Bergman (1990), as much as 25 to 40% of the potential energy content of carbohydrates entering the large intestine was used for microbial processes or lost as methane. The increase in the efficiency of energy utilization in this study would be small. The ileal energy digestibility in wheat was relatively high to start with (93.1%, Table 3-7). Micronization would be expected to have a larger

effect in cereals, for example barley, in which the ileal energy digestibility is lower (Li et al., 1996). Starch escaping digestion in the small intestine of pigs fed the experimental diets was completely fermented in the large intestine, which was also reported by Sauer et al. (1977) in studies with wheat and by Fadel et al. (1989) in studies with hulless barley.

There was a larger disappearance of the total of the dispensable than indispensable amino acids (Table 3-6). The reason for this effect has been previously discussed by Li et al. (1994). There was net synthesis of methionine in the large intestine of pigs fed the W + SBM diet. Net synthesis of methionine has also been reported in other studies (e.g., Li and Sauer, 1994).

The apparent ileal digestibilities of amino acids in ground and micronized wheat (Table 3-7) were determined with the difference method. Fan and Sauer (1995) previously showed that amino acid digestibilities in ingredients low in CP (amino acid) content should be determined with the difference rather than direct method which was illustrated in studies with barley that contained 11.4% CP. Of the indispensable amino acids, digestibility values in barley were lower when they were determined with the direct method, ranging from 2.4 (histidine) to 9.1 (threonine) percentage units. The CP content of wheat in this study was 11.5%. As was pointed out by Fan et al. (1994), the apparent digestibility values of amino acids in a feedstuff are dependent on their respective amino acid levels and reach a plateau when their threshold levels are reached. Threshold levels in feedstuffs low in amino acid content can only be reached when the difference method is used.

Micronization improved the ileal digestibilities of all amino acids except for methionine and cysteine. Of the indispensable amino acids, the differences ranged from 2.2

(arginine) to 12.2 (threonine) percentage units; the differences were significant ($P < .05$) for histidine, lysine, phenylalanine and threonine. Of the dispensable amino acids, the differences ranged from 4.9 (tyrosine) to 17.2 (glycine) percentage units; the differences were significant ($P < .05$) for alanine, aspartic acid, glutamic acid, serine and tyrosine.

As has been reported in many other studies, of the indispensable amino acids, the digestibilities of lysine and threonine were relatively low, 57.6 and 69.7 % for lysine, and 64.7 and 76.9% for threonine in ground and micronized wheat (Table 3-7), respectively. The probable reasons for the relatively low lysine digestibility in wheat were reviewed by Mosenthin et al. (1997). The digestibility values of lysine and threonine, the first and second limiting amino acids in wheat, respectively, fall within the range of values reported by Mosenthin et al. (1997). These authors reported digestibility values of 73 (± 6.5) and 72 (± 6.7) % for lysine and threonine, respectively. In the same order for these amino acids, the minimum and maximum values ranged from 62 to 84 and 51 to 78%, respectively. Furthermore, the considerable variation in amino acid digestibility values in wheat results from several factors, including fineness of grinding, as was reviewed by Sauer and Ozimek (1986). In addition, digestibility values are affected by method of determination i.e. the direct versus the difference method (Fan and Sauer, 1994); the direct method was used in nearly all studies reported in the literature. Furthermore, BW (age) may affect digestibility values (Li et al., 1993; Li and Sauer, 1994). The present studies were carried out with pigs over the BW range from 7.0 to 16.5 kg. Most studies on wheat have been carried out with growing and finishing pigs.

Micronization of wheat also increased ($P < 0.05$) the ileal digestibility of starch from

93.1 to 99.3% (Table 3-7). The ileal digestibilities of starch in the W + SBM and MW + SBM diets (Table 3-4) were considered to be similar to ground and micronized wheat, respectively, since soybean meal contributed only a negligible amount (.6%) of starch to the total dietary starch content.

Micronization of wheat improved ($P < .05$) the ileal lysine digestibility from 57.6 to 69.7% (Table 7), which indicated indirectly that Maillard reactions did not occur. According to Noguchi et al. (1982) and Björck et al. (1983), a high moisture content during heat-processing will inhibit the Maillard reaction and improve lysine digestibility. This effect can be explained by the law of mass action, as water is produced in the reversible phase of Maillard reactions.

In conclusion, micronization of wheat improved the ileal digestibilities of most of the amino acids, including lysine and threonine. Micronization also shifted the disappearance of starch from the large to the small intestine, which may result in an improvement in the efficiency of energy utilization.

Table 3-1. Formulation (%) of the experimental diets

Ingredients	Diets [z]		
	W + SBM	MW + SBM	C + SBM
Wheat	72.36	-	-
Micronized wheat	-	72.36	-
Soybean meal	20.42	20.42	37.12
Corn starch[y]	-	-	55.31
Canola oil	3.00	3.00	3.00
Biophos[x]	1.20	1.20	1.95
Calcium carbonate	1.32	1.32	.92
Trace-mineralized salt[w]	.30	.30	.30
Vitamin-mineral premix[v]	1.00	1.00	1.00
Antibiotics[u]	.10	.10	.10
Chromic oxide	.30	.30	.30

[z] W + SBM: ground wheat-soybean meal diet; MW + SBM: micronized wheat-soybean meal diet; C + SBM: corn starch-soybean meal diet.

[y] St. Lawrence Starch Company Ltd., Mississauga, ON, Canada.

[x] Provided (%): available phosphorous, 15 - 18 and calcium, 24. Supplied by Continental Lime Ltd., Exshaw, AB, Canada.

[w] Windsor Salt Co., Toronto, ON, Canada. Composition (%): NaCl, 96.5; ZnO, .4; $FeCO_3$, .16; MnO, .12; CuO, .033; $Ca(IO_3)_2$, .007; CaO, .004.

[v] Provided the following (per kg diet): vitamin A, 10,000 IU; vitamin D_3, 1,000 IU; vitamin E, 80 IU; vitamin $_K$, 2.0 mg; vitamin B_{12}, .03 mg; riboflavin, 12 mg; niacin, 40 mg; d-pantothenic acid, 25 mg ; choline, 1,000 mg; d-biotin, .25 mg; folic acid, 1.6 mg; thiamine, 3.0 mg; Ethoxyquin, 25 mg; pyridoxine, 2.25 mg. Fe, 150 mg; Zn, 150 mg; Cu, 125 mg; I, .21 mg; Se, .3 mg. Supplied by Hoffmann-LaRoche Ltd., 2455 Meadowpine Blvd., Mississauga, ON, Canada.

[u] Veterinary LS-20 premix, provided (g/kg mixture): Lincomycin hydrochloride 22, Spectinomycin sulphate 22. Supplied by the Upjohn Company, Animal Health Division, Orangeville, ON, Canada.

Table 3-2. Dry matter, neutral-detergent fiber, energy, starch, crude protein and amino acid content (%)[z] of the dietary ingredients

	Ingredients[y]		
Item	W	MW	SBM
Dry matter	87.6	88.7	88.7
Neutral-detergent fiber	11.0	10.4	7.1
Energy (MJ kg^{-1})	18.4	18.5	19.7
Starch	76.9	74.4	1.7
Crude protein	13.1	13.3	55.0
Amino acids			
Indispensable			
Arginine	.59	.59	3.58
Histidine	.30	.30	1.35
Isoleucine	.49	.51	2.41
Leucine	.94	.95	3.92
Lysine	.34	.34	3.25
Methionine	.25	.24	.68
Phenylalanine	.60	.62	2.55
Threonine	.37	.36	1.82
Valine	.60	.63	2.49
Dispensable			
Alanine	.50	.53	2.53
Aspartic acid	.72	.73	5.76
Cysteine	.31	.30	.54
Glutamic acid	4.15	4.36	9.76
Glycine	.64	.57	1.91
Serine	.60	.63	2.51
Tyrosine	.30	.30	1.72

[z] Dry matter basis

[y] W: ground wheat; MW: micronized wheat; SBM: soybean meal.

Table 3-3. Dry matter, neutral-detergent fiber, energy, starch, crude protein and amino acid content (%)[z] of the experimental diets

	Diets[y]		
Item	W + SBM	MW + SBM	C + SBM
Dry matter	89.5	89.8	90.7
Neutral detergent fiber	9.2	8.9	2.6
Gross energy (MJ kg^{-1})	18.7	18.9	18.5
Starch	54.9	53.5	58.9
Crude protein	20.4	20.6	19.8
Amino acids			
Indispensable			
Arginine	1.14	1.14	1.30
Histidine	.48	.49	.50
Isoleucine	.84	.85	.88
Leucine	1.45	1.47	1.42
Lysine	.89	.90	1.18
Methionine	.31	.31	.23
Phenylalanine	.94	.96	.93
Threonine	.63	.62	.66
Valine	.94	.96	.63
Dispensable			
Alanine	.87	.89	.91
Aspartic acid	1.68	1.69	2.09
Cysteine	.32	.32	.18
Glutamic acid	4.92	5.09	3.54
Glycine	.84	.80	.69
Serine	.94	.96	.91
Tyrosine	.56	.56	.63

[z] Dry matter basis.

[y] Refer to Table 3-1.

Table 3-4. Apparent ileal digestibilities (%) of dry matter, energy, starch, crude protein and amino acids in the experimental diets

Item	Diets[z]			SEM[y]
	W + SBM	MW + SBM	C + SBM	
Dry matter	56.7[c]	67.4[b]	76.0[a]	1.29
Energy	59.3[c]	70.0[b]	79.5[a]	1.25
Starch	92.7[b]	99.1[a]	98.4[a]	.60
Crude protein	76.2	76.6	78.1	.92
Amino acids				
Indispensable				
Arginine	87.7[c]	90.2[b]	92.3[a]	.58
Histidine	85.8[b]	89.8[a]	90.8[a]	.50
Isoleucine	83.3[b]	87.5[a]	87.5[a]	.61
Leucine	84.1[b]	88.2[a]	86.7[a]	.67
Lysine	79.8[c]	84.4[b]	88.1[a]	.82
Methionine	81.9	80.5	82.4	.90
Phenylalanine	84.8[b]	88.6[a]	87.6[a]	.64
Threonine	71.4[b]	80.4[a]	78.9[a]	.98
Valine	80.6[b]	86.0[a]	85.7[a]	.79
Dispensable				
Alanine	77.2[b]	83.5[a]	85.2[a]	.75
Aspartic acid	81.3[b]	85.3[a]	86.7[a]	.68
Cysteine	74.5	72.6	66.4	2.42
Glutamic acid	89.2[b]	93.5[a]	89.2[b]	.77
Glycine	69.8	79.3	74.0	2.37
Serine	82.2[b]	87.2[a]	86.2[a]	.70
Tyrosine	82.5[b]	87.2[a]	88.5[a]	.58

[z] Refer to Table 3-1.

[y] Standard error of the mean

[a,b,c] Means in the same row with different superscript letters differ ($P < .05$)

Table 3-5. Apparent faecal digestibilities (%) of dry matter, energy, starch, crude protein and amino acids in the experimental diets

Item	Diets[z]			SEM[y]
	W + SBM	MW + SBM	C + SBM	
Dry matter	86.2[c]	87.3[b]	93.0[a]	.27
Energy	86.0[c]	87.6[b]	94.4[a]	.36
Starch	100.0	100.0	100.0	.00
Crude protein	85.1[c]	86.8[b]	89.5[a]	.82
Amino acids				
Indispensable				
Arginine	93.5[b]	94.2[b]	96.4[a]	.42
Histidine	91.2[b]	91.7[b]	94.5[a]	.56
Isoleucine	86.2[b]	88.2[b]	91.4[a]	.93
Leucine	89.8[b]	90.6[ab]	92.7[a]	.68
Lysine	86.7[b]	88.2[b]	94.0[a]	.83
Methionine	77.4[b]	81.5[a]	82.3[a]	1.20
Phenylalanine	89.6[b]	90.5[ab]	92.6[a]	.69
Threonine	83.2[b]	85.1[b]	89.3[a]	1.08
Valine	86.4[b]	88.2[ab]	90.9[a]	.94
Dispensable				
Alanine	85.4[b]	86.9[b]	91.5[a]	.75
Aspartic acid	88.4[b]	89.0[b]	94.7[a]	.38
Cysteine	86.1	87.7	86.4	.82
Glutamic acid	96.1	96.3	96.7	.17
Glycine	86.6	87.4	89.0	1.00
Serine	90.3[b]	91.8[b]	93.8[a]	.56
Tyrosine	88.9[b]	91.3[ab]	93.3[a]	.91

[z] Refer to Table 3-1.

[y] Standard error of the mean

[a-c] Means in the same row with different superscript letters differ ($P < .05$)

Table 3-6. Disappearance[z] of dry matter, energy, starch, crude protein and amino acids in the large intestine of pigs fed the experimental diets

Item	Diets[y]			SEM[x]
	W + SBM	MW + SBM	C + SBM	
Dry matter	215.3[a]	174.4[b]	166.7[b]	11.95
Energy (MJ kg^{-1} DMI)	5.0[a]	3.3[b]	2.8[c]	.60
Starch	39.6[a]	4.8[b]	9.1[b]	3.42
Crude protein	18.2	21.1	22.6	2.22
Amino acids				
Indispensable				
Arginine	.66	.47	.53	.08
Histidine	.26[a]	.10[b]	.18[ab]	.03
Isoleucine	.24	.06	.34	.09
Leucine	.82	.36	.85	.14
Lysine	.61[a]	.29[b]	.70[a]	.10
Methionine	-.14	.03	.00	.04
Phenylalanine	.45[a]	.18[b]	.46[a]	.09
Threonine	.74[a]	.29[b]	.68[a]	.08
Valine	.55	.21	.33	.11
Dispensable				
Alanine	.72[a]	.30[b]	.58[a]	.09
Aspartic acid	1.19[b]	.63[c]	1.67[a]	.14
Cysteine	.37	.48	.36	.07
Glutamic acid	3.40[a]	1.43[b]	2.66[a]	.07
Glycine	1.41[a]	.65[b]	1.04[ab]	.19
Serine	.76[a]	.44[b]	.69[a]	.20
Tyrosine	.36	.23	.30	.05

[z] g kg^{-1} DMI

[y] Refer to Table 3-1.

[x] Standard error of the mean.

[a,b,c] Means in the same row with different superscript letters differ ($P < .05$).

Table 3-7. Effect of micronization of wheat on the apparent ileal digestibilities (%) of starch, crude protein and amino acids

Item	W[z]	MW[z]	SEM[y]
Starch	93.1	99.3	.71
Crude protein	73.6	75.3	1.92
Amino acids			
Indispensable			
Arginine	80.3	82.5	1.05
Histidine	79.4[b]	85.8[a]	.59
Isoleucine	80.1	83.9	1.08
Leucine	82.7	87.4	1.14
Lysine	57.6[b]	69.7[a]	1.86
Methionine	64.7	62.2	1.59
Phenylalanine	81.7[b]	87.0[a]	.98
Threonine	64.7[b]	76.9[a]	1.54
Valine	79.0	83.8	1.40
Dispensable			
Alanine	69.5[b]	78.1[a]	1.49
Aspartic acid	71.6[b]	79.3[a]	1.56
Cysteine	70.0	66.6	3.56
Glutamic acid	89.6[b]	94.9[a]	.09
Glycine	65.5	82.7	.65
Serine	80.0[b]	85.5[a]	.93
Tyrosine	73.0[b]	77.9[a]	.92

[z] Refer to Table 3-2.

[y] Standard error of the mean.

[a,b] Means in the same row with different superscript letters differ ($P < .05$).

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CHAPTER 4

NUTRITIVE VALUE OF WHEAT SHORTS AND FACTORS AFFECTING THE DIGESTIBLE AMINO ACID CONTENT FED TO GROWING PIGS

A. Introduction

Wheat by-products are produced when wheat is processed into flour for human consumption. Wheat bran and shorts are used as ingredients in diets for swine. Wheat shorts comprise the layer of the wheat kernel just inside the outer bran covering endosperm and bran particles.

The greatest problem associated with the use of the wheat shorts in diets for swine is the lack of uniformity due to variable proportions of endosperm and bran particles. A certain proportion of wheat bran is always included in wheat shorts; an increase in this proportion will decrease the digestible energy content of shorts and alter its amino acid profile. Wheat shorts usually contain 5 to 10% crude fiber and 15 to 20% crude protein (CP). The inclusion of shorts in diets for growing-finishing pigs has been recommended at up to 30% (Holden and Zimmerman, 1991). At such high levels of inclusion, wheat shorts contribute significantly to the energy and amino acid intake. Thus, an accurate estimation of the digestibility coefficients is very important to effectively use this ingredient in the formulation of diets.

There is a scarcity of information on the ileal amino acid digestibilities in wheat shorts and results available show considerable variation. Furthermore, there is no information

on the effect of inherent factors in wheat shorts on amino acid digestibility. Recent studies with wheat have shown that, in addition to approaches in methodology, differences in fiber content (neutral-detergent fiber, NDF) were, in part, responsible for this variation (Fan, 1994).

The objectives of these studies were to investigate the variation in apparent ileal digestibilities of amino acids in different samples of wheat shorts and to identify factors responsible for the variation. Five samples of wheat shorts differing in fiber content were created for these studies. These five samples, hereafter referred to as wheat fractions, contained different proportions of bran, flour and shorts which provided a wide range in the content of NDF.

B. Experimental Procedures

Animals and diets

Six barrows (Camborough × Canabrid), average initial body weight (BW) 37.2 kg, were obtained from the University of Alberta swine herd and housed individually in stainless steel metabolism crates in a temperature-controlled barn (20 to 22 °C). The pigs were fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer et al. (1983). The cannulas were modified according to De Lange et al. (1989). During a 14-d recuperation period, the barrows were fed a 16% CP grower diet (Sauer et al., 1983). A detailed description of pre- and post-operative care was previously presented by Li et al. (1994).

After recuperation, the barrows were fed one of six experimental diets (Table 4-1)

according to a 6 × 6 Latin square design. They were fed twice daily, equal amounts each meal, at 0800 and 1600. During the first experimental period, the daily dietary allowance was provided at a rate of 5% (wt/wt) of the average BW determined at the initiation of the experiment. Thereafter, the daily dietary allowance was increased by 100 g at each following experimental period. Water was freely available from a low-pressure drinking nipple. At the conclusion of the experiment, the barrows, average final BW 90.3 kg, were sacrificed and dissected to determine whether cannulation had caused intestinal abnormalities.

Six diets were formulated (Table 4-1) to contain 17% CP (%N × 6.25, as-fed basis). Diets A, B, C, D and E contained 17.53% soybean meal (SBM), which contributed 50% CP to these diets. The wheat fractions contained wheat shorts (WS), wheat bran (WB) or wheat flour (WF) alone or in combination, which contributed the remaining 50% CP to these diets. Diet F contained 35.05% SBM, which was the sole source of dietary CP. The proportions of WS, WB and WF in the wheat fractions were 70% WS and 30% WB in diet A, 85% WS and 15% WB in diet B, 100% WS in diet C, 85% WS and 15% WF in diet D and 70% WS and 30% WF in diet E. Dextrose (10%) was included in the diets to improve palatability. Canola oil was included at a level of 3% to reduce the dustiness of the diets. Vitamins and minerals were supplemented to meet or exceed NRC (1988) standards. Chromic oxide (.3%) was included in the diet as the digestibility marker.

Each experimental period comprised 12 d. The collection of feces was initiated at 0800 on d 8 of each experimental period and continued for 48 h. Ileal digesta were collected for a total of 24 h; from 0800 to 1000 on d 10 and at 2-h intervals thereafter until 0600 on d 11 and from 1800 to 2000 on d 11 and at 2-h intervals thereafter until 1600 on d 12. The

procedure for the collection of digesta was adapted from Li (1992). Feces and digesta were frozen at -28 °C immediately after collection. The samples were pooled within barrows and periods. The animals used in this experiment were cared for in accordance with the guidelines established by CCAC (1993) and approved by the Faculty of Agriculture, Forestry and Home Economics Animal Care Committee of the University of Alberta.

Chemical and statistical analysis

Samples of the diets were taken each time the meal allowances were weighed out and finally pooled for each dietary treatment. Samples of diets, feces and digesta were freeze-dried and ground in a Wiley mill through .8-mm mesh before analyses. Analyses for dry matter (DM) and organic matter (OM) were carried out according to AOAC (1984). Gross energy (GE) was determined using a Parr 1241 Adiabatic Bomb Calorimeter (Parr Instruments, Moline, IL). Crude protein was determined with a Leco FP-428 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Analyses of NDF, acid-detergent fiber (ADF) and lignin in the dietary ingredients were carried out according to principles outlined by Goering and Van Soest (1970). The contents of starch in the dietary ingredients, digesta and feces were determined with the Megazyme total starch analysis procedure (thermostable α - amylase/amyloglucosidase method; Megazyme, Warriewood, Australia). Chromic oxide was measured according to Fenton and Fenton (1979).

For amino acid analyses, except for cysteine and methionine, approximately 0.1 g of sample was weighed into a screw-capped test tube and mixed with 3 ml of 6 N HCL. The tubes were purged with nitrogen and then hydrolyzed in an oven at 110 °C for 24 h. The hydrolyzed samples were mixed with the internal standard, DL-amino-n-butyric acid, and

centrifuged at 1,110 × g for 15 min at 4°C. The supernatant of the sample was analyzed according to principles outlined by Jones and Gilligan (1983) using a Varian 5000 high performance liquid chromatography system with a reverse-phase column and a Varian Fluorichrom detector (Varian Canada Inc., Mississauga, ON). This procedure was described in more detail by Sedgwick et al. (1991). The amino acids were derivatized with an o-phthaldialdehyde reagent solution. The mobile phase consisted of two solvents with a flow rate of 1.1 ml/min. Solvent A contained .1 M sodium acetate (pH 7.2), methanol, and tetrahydrofuran in a ratio of 90 to 5; solvent B was pure methanol. Peaks were recorded and integrated using the Ezchrom™ Chromatography Data System (version 2.12; Shimadzu Scientific Instruments Inc., Columbia, MD). Methionine and cysteine were determined as methionine sulfone and cysteic acid after oxidation with 98% performic acid overnight according to AOAC (1984). The oxidized samples were dried according to procedures described by Dugan et al. (1992), then hydrolyzed and analyzed in the same manner as the other amino acids. Tryptophan and proline were not measured.

Analyses of ingredients and diets were carried out in triplicate; digesta and feces in duplicate.

The apparent ileal and fecal digestibility values of the experimental diets and the apparent ileal digestibility values of the wheat fractions were subjected to statistical analysis using the General Linear Model Procedure (GLM) of SAS (1990) for a 6 × 6 Latin square design. The apparent ileal CP and amino acid digestibilities of the wheat fractions were determined with the difference method (Fan and Sauer, 1995).

Means of dietary treatments, experimental periods and barrows were compared using

the Student-Newman Keul's multiple range test. Pearson partial correlation analyses were conducted to determine the relationship between the NDF content and apparent ileal digestibility values of CP and amino acids of the wheat fractions. Variation contributed by barrows and periods were removed and correlation coefficients were computed from the Error SS&CP (df = 19) using the repeated measurement option of GLM of SAS (1990). The regression equations were developed using the Regression Procedure of SAS (1990).

C. Results and Discussion

The pigs remained healthy and consumed their daily allowances throughout the experiment. Postmortem examinations, conducted at the conclusion of the experiment, revealed no adhesions or other intestinal abnormalities

The chemical and amino acid composition of the experimental diets and ingredients are presented in Tables 4-2 and 4-3, respectively. The contents of NDF, ADF and lignin in the experimental diets were calculated from the analyzed values in SBM, WB, WS, and WF. The contents of NDF, ADF and lignin decreased gradually from diet A to E. The analyzed values of CP and amino acids in the experimental diets were very close to the calculated values based on the analyzed values in SBM, WB, WS, and WF.

The apparent ileal digestibility values of DM, GE, CP and amino acids in the experimental diets are presented in Table 4-4. Among the experimental diets containing the wheat fractions, the digestibilities were usually lowest in diet A (15.3% WB and 35.7% WS) and highest in diets D (44.1% WS and 7.8% WF) and E (37.2% WS and 15.9% WF) which contained no WB. As expected, the digestibilities were highest in diet F, a corn starch-based

SBM diet. The apparent ileal amino acid digestibility values in SBM are within the range of values reported by Sauer and Ozimek (1986) and Knabe (1991).

The changes in the proportions of WB to WS and WF in the wheat fractions of the experimental diets from A to E resulted in an increase ($P < .05$) in the ileal digestibilities of DM and GE which corresponded with a decrease in fiber content in these diets (Table 4-4). These results are in agreement with those reported by Fernandez and Jørgensen (1986) who reported a decrease in the ileal digestibility of GE as the crude fiber content in the diet was increased. They also reported an enhanced fermentation of energy in the large intestine when the fiber level of the diet was increased. There were no differences ($P > .05$) in the ileal digestibility of starch among the experimental diets; the digestibility values ranged from 97.6 to 98.7% (Table 4-4). These values are higher than the ileal digestibility values of starch in wheat fed to young pigs, which was 92.7% (Chapter 3). These results suggest that the maturity of the digestive tract may be an important factor influencing the digestibility of starch.

There was an increase in the ileal digestibility of CP and amino acids with a decrease in the fiber content of the experimental diets (Table 4-4). The CP digestibilities in diets A to E were 11.3 to 6.6 percentage units lower than those in diet F. Graham et al. (1986) reported that the dietary inclusion of wheat bran depressed the ileal digestibility of nitrogen. In studies with NDF purified from wheat bran, the ileal nitrogen digestibility decreased by 4.9 percentage units when 18% was included in the diet (Schulze et al., 1994) and by 5.2 percentage units when 15% was included in the diet (Lenis et al., 1996). The lower ileal digestibilities of CP with increasing dietary fiber content may be attributed to an increase in

the rate of passage of digesta (Stanogias and Pierce, 1985), adsorption of amino acids and peptides to fiber, increased losses of endogenous nitrogen and a lower digestibility of CP associated with fiber (Donangelo and Eggum, 1985).

Within each experimental diet, compared with the other amino acids, the apparent ileal digestibility of arginine was usually relatively high whereas those of threonine and glycine were relatively low (Table 4-4). The relatively high apparent ileal digestibility of arginine and low digestibility of threonine in these studies tend to support the hypothesis that enzyme specificity is an important determinant of apparent amino acid digestion and absorption in the small intestine. As was discussed by Low (1980), of the indispensable amino acids, arginine would be expected to appear first after enzymatic hydrolysis and threonine last based on the specificity of the proteases and peptidases involved. The relatively low apparent ileal digestibilities of threonine and glycine may, in part, result from their relatively high concentrations in endogenous secretions. Studies by Holmes et al. (1974) and Sauer et al. (1977) showed a relatively high content of threonine and glycine in digesta collected from the distal ileum of growing pigs fed a protein-free diet. Glycine, a major constituent base of the bile salt conjugates, accounts for 90% of the total of the amino acids secreted in porcine bile juice (Souffrant, 1991). The bile salt conjugates are degraded in the distal ileum by intestinal bacteria; 90% of the bile salts are re-absorbed via active transport and enter the enterohepatic circulation. However, the deconjugated glycine escapes re-absorption and enters the large intestine (Shiau, 1987; Newsholme and Leech, 1984). Furthermore, the small intestinal secretions, which include mucin, supply the largest proportion of nitrogen to the endogenous nitrogen secretions in the small intestine (Auclair,

1986). As was shown by Neutra and Forstner (1987), 'native' mucin which represents over 95% of mucin glycoprotein, is very rich in threonine in addition to serine and proline. The low apparent ileal digestibility of threonine may also, in part, result from its relatively low rate of absorption. Buraczewska (1979) studied the ability of different segments of the small intestine to absorb amino acids and peptides, using temporarily-isolated loops in growing-finishing pigs. Of the indispensable amino acids, using segments of the middle and distal small intestine, the rates of absorption were highest for arginine, methionine, isoleucine and leucine and lowest for threonine and histidine.

The fecal digestibilities of DM, GE and CP increased from diet A to diet E, which follow the decrease in fiber content among the experimental diets (Table 4-5). Starch escaping digestion in the small intestine was completely fermented in the large intestine which was also reported in other studies (e.g., Sauer et al., 1977; Fadel et al., 1989). It is not possible to determine the digestibility of GE in the wheat fractions as such with the difference method. However, the digestibilities of GE in the experimental diets should reflect those of the constituent wheat fractions. Batterham et al. (1980) also reported considerable variation in the digestibility of GE in wheat by-products which they attributed to different rates of extraction of bran at different mills. Fernandez and Jørgensen (1986) reported a one percentage unit decrease in the digestibility of GE for each one percentage unit increase in NDF content of the diet.

The apparent fecal digestibilities of the amino acids are also presented in Table 4-5. However, as is well accepted at present that, because of the modifying action of the microflora in the large intestine, ileal rather than fecal digestibility values should be used in

diet formulation (e.g., Sauer and Ozimek, 1986; Knabe, 1991).

The disappearance of DM, starch, GE, CP and amino acids, expressed in percentage units or quantitatively as g per kg dry matter intake (DMI), are presented in Tables 4-6 and 4-7, respectively. Expressed as percentage units, the disappearance was relatively large for threonine, cysteine, glycine and serine. The disappearance of these amino acids in the large intestine usually exceeded 10 percentage units. Expressed quantitatively, as g per kg DMI, of the indispensable amino acids, the disappearance was relatively large for leucine and threonine; of the dispensable amino acids the disappearance was relatively large for aspartic acid, glutamic acid and glycine, which is in agreement with other reports (e.g., Sauer et al., 1991; Li et al., 1994).

The apparent ileal digestibilities of CP and amino acids in the wheat fractions are presented in Table 4-8. These values were calculated with the difference method according to Fan and Sauer (1995). The digestibilities were usually lower in wheat fractions A, B and C than in D and E. Of the indispensable amino acids within each wheat fraction, the digestibilities were usually highest for arginine, histidine and methionine and lowest for lysine and threonine, which are often first- and second-limiting in diets for pigs. The digestibilities of lysine ranged from 54.7 to 64.1%, while the digestibilities of threonine ranged from 48.9 to 69.2%. These values compare favourably to those reported by Sauer et al. (1977) for wheat offal in which the apparent ileal digestibilities of lysine and threonine were 66.4 and 54.0 %, respectively.

The NDF content in the wheat fractions ranged from 29.5 to 42.3% (by calculation from Tables 4-1, 4-2 and 4-3). Simple linear relationships were established between the

apparent ileal digestibilities of CP and amino acids and the NDF content in the wheat fractions (Table 4-9). With the exception of arginine, lysine, methionine, cysteine and tyrosine, there were significant ($P < .05$) negative correlations between the apparent ileal amino acid digestibilities and the NDF content in the wheat fractions. Fan et al. (1996), in a study with canola meal fed to pigs, also reported negative correlations between the apparent ileal amino acid digestibilities (with the exception of arginine) and NDF content. A detailed discussion on the effect of NDF on amino acid digestibility, including the effect on endogenous amino acid losses, is presented in Chapter 5.

In conclusion, there were considerable differences in the apparent ileal digestibility values of amino acids among the different wheat fractions. Differences in NDF content were, in part, responsible.

Table 4-1. Formulation (%) of the experimental diets

Ingredients	Diets					
	A	B	C	D	E	F
Soybean meal	17.53	17.53	17.53	17.53	17.53	35.05
Wheat shorts	35.73	43.71	50.66	44.08	37.19	.00
Wheat bran	15.32	7.63	-	-	-	-
Wheat flour	-	-	-	7.78	15.94	-
Corn starch[a]	15.45	15.19	15.87	14.52	13.15	48.31
Dextrose[b]	10.00	10.00	10.00	10.00	10.00	10.00
Canola oil	3.00	3.00	3.00	3.00	3.00	3.00
Biophos	-	-	-	.25	.45	1.32
Calcium carbonate	1.37	1.34	1.34	1.24	1.15	.72
Trace-mineralized salt[c]	.30	.30	.30	.30	.30	.30
Vitamin-mineral premix[d]	1.00	1.00	1.00	1.00	1.00	1.00
Chromic oxide[e]	.30	.30	.30	.30	.30	.30

[a] St. Lawrence Starch Company Ltd., Mississauga, ON.

[b] Corn Products, Englewood Cliffs, NJ.

[c] Windsor Salt Co., Toronto, ON. Composition (%): NaCl, 96.5; ZnO, .4; $FeCO_3$, .16; MnO, .12; CuO, .033; $Ca(IO_3)_2$, .007; CaO, .004.

[d] Provided the following (per kilogram of diet): vitamin A, 10,000 IU; vitamin D_3, 1,000 IU; vitamin E, 80 IU; vitamin K_3, 2.0 mg; vitamin B_{12}, .03 mg; riboflavin, 12 mg; niacin, 40 mg; d-pantothenic acid, 25 mg; choline, 1,000 mg; d-biotin, .25 mg; folic acid, 1.6 mg; thiamin, 3.0 mg; Ethoxyquin, 5.0; pyridoxine, 2.25 mg. Fe, 150 mg; Zn, 150 mg; Cu, 125 mg; I, .21 mg; Se, .3 mg. Supplied by Hoffmann-LaRoche Ltd., 2455 Meadowpine Blvd., Mississauga, ON, Canada.

[e] Fisher Scientific, Fair Lawn, NJ.

Table 4-2. Chemical and amino acid composition (%) of the experimental diets[a]

Item	Diets					
	A	B	C	D	E	F
Dry matter	89.9	90.0	90.2	90.4	90.7	90.6
Organic matter	93.4	93.4	93.6	93.8	94.0	94.6
Starch	9.1	9.2	9.1	13.8	18.8	50.8
Energy (kcal/kg)	4506.5	4549.0	4539.7	4527.8	4507.2	4372.2
Neutral-detergent fiber	24.2	24.1	23.6	20.8	18.0	2.5
Acid-detergent fiber	8.4	8.4	8.2	7.3	6.3	.4
Lignin	5.8	5.8	5.6	4.9	4.2	.2
Crude protein	18.9	19.0	18.9	18.7	18.5	18.8
Amino acids						
Indispensable						
Arginine	1.22	1.21	1.20	1.16	1.12	1.24
Histidine	.47	.47	.47	.46	.45	.46
Isoleucine	.72	.72	.72	.72	.72	.83
Leucine	1.23	1.23	1.22	1.22	1.23	1.34
Lysine	.89	.89	.89	.86	.83	1.11
Methionine	.27	.27	.26	.26	.26	.23
Phenylalanine	.79	.79	.79	.80	.81	.88
Threonine	.64	.64	.63	.62	.61	.69
Valine	.87	.88	.87	.86	.85	.88
Dispensable						
Alanine	.99	.99	.98	.95	.92	.93
Aspartic acid	1.64	1.64	1.63	1.59	1.55	2.00
Cysteine	.38	.38	.38	.37	.37	.27
Glutamic acid	3.37	3.37	3.33	3.51	3.69	3.20
Glycine	.84	.84	.83	.81	.79	.73
Serine	.79	.80	.78	.78	.79	.95
Tyrosine	.34	.34	.34	.34	.34	.39

[a] Dry matter basis

Table 4-3. Chemical and amino acid composition[a] (%) of the ingredients in the experimental diets

Item	Ingredients			
	Soybean meal	Wheat bran	Wheat shorts	Wheat flour
Dry matter	88.3	94.5	96.3	91.1
Energy (kcal/kg)	4707.3	4601.6	4625.5	4449.6
Starch	1.7	16.8	17.0	79.8
Neutral-detergent fiber	7.1	45.1	41.2	2.1
Acid-detergent fiber	3.8	14.5	14.0	.8
Lignin	.4	11.4	10.3	.5
Crude protein	55.0	17.3	17.4	15.6
Amino acids				
Indispensable				
Arginine	3.64	1.14	1.06	.53
Histidine	1.34	.46	.44	.31
Isoleucine	2.44	.56	.55	.55
Leucine	3.92	1.03	1.01	1.01
Lysine	3.25	.59	.61	.22
Methionine	.78	.28	.28	.23
Phenylalanine	2.57	.67	.64	.75
Threonine	2.02	.54	.53	.38
Valine	2.57	.80	.79	.63
Dispensable				
Alanine	2.72	.96	.95	.51
Aspartic acid	5.87	1.16	1.15	.60
Cysteine	.78	.46	.45	.38
Glutamic acid	9.38	3.31	3.19	5.19
Glycine	2.15	.90	.86	.51
Serine	2.49	.67	.65	.66
Tyrosine	1.14	.26	.27	.27

[a] Dry matter basis

Table 4-4. Apparent ileal digestibilities (%) of dry matter, starch, energy, crude protein, and amino acids in the experimental diets

Item	Diets						SEM[a]
	A	B	C	D	E	F	
Dry matter	55.3[b]	55.3[b]	58.3[c]	61.8[d]	62.9[d]	82.0[e]	.80
Starch	98.1	97.6	97.9	98.2	98.7	100.0	.09
Energy	60.2[b]	60.2[b]	63.0[b]	65.6[c]	66.5[c]	84.7[d]	.80
Crude protein	70.6[b]	71.6[bc]	72.4[bcd]	75.3[cd]	74.8[d]	81.9[e]	.91
Amino acids							
Indispensable							
Arginine	83.5[b]	85.1[c]	85.9[cd]	87.2[d]	86.1[cd]	90.9[e]	.48
Histidine	79.6[b]	80.9[bc]	81.7[bcd]	83.9[cd]	83.0[d]	87.3[e]	.74
Isoleucine	72.9[b]	76.8[c]	78.0[cd]	80.6[de]	79.7[d]	82.3[e]	.75
Leucine	74.2[b]	77.5[c]	78.6[cd]	81.2[de]	80.4[e]	83.5[f]	.72
Lysine	74.6[b]	76.2[b]	77.2[b]	78.4[b]	76.6[b]	86.2[c]	.96
Methionine	79.5[b]	81.8[bc]	81.2[b]	83.3[c]	82.8[bc]	87.2[d]	.82
Phenylalanine	75.0[b]	79.2[c]	79.3[c]	82.1[d]	81.8[d]	84.1[e]	.61
Threonine	62.1[b]	65.8[bc]	68.2[cd]	71.3[d]	70.7[d]	73.0[d]	1.31
Valine	70.8[b]	74.9[bc]	75.9[bc]	78.6[c]	77.5[c]	79.3[d]	.90
Dispensable							
Alanine	69.4[b]	74.0[c]	75.4[c]	77.4[c]	76.0[c]	84.2[d]	1.52
Aspartic acid	69.5[b]	74.3[c]	75.5[c]	77.8[c]	76.7[c]	81.4[d]	.98
Cysteine	65.6[b]	66.5[bc]	63.8[b]	72.3[d]	67.9[bc]	69.9[bc]	1.53
Glutamic acid	82.3[b]	84.0[bc]	86.2[cd]	87.6[cd]	87.6[d]	87.0[bc]	.84
Glycine	63.4[b]	66.2[bc]	68.1[bcd]	72.3[de]	70.1[cd]	76.0[e]	1.36
Serine	71.6[b]	74.9[c]	75.5[c]	78.7[c]	77.8[c]	82.4[d]	.98
Tyrosine	66.1[b]	68.3[bc]	69.1[bc]	73.9[c]	70.8[bc]	75.5[c]	1.71

[a] Standard error of the mean

[b,c,d,e,f] Means in the same row with different superscript letters differ ($P < .05$)

Table 4-5. Apparent fecal digestibilities (%) of dry matter, starch, energy, crude protein, and amino acids in the experimental diets

Item	Diets						SEM[a]
	A	B	C	D	E	F	
Dry matter	73.2[b]	73.9[b]	76.2[c]	78.2[d]	80.3[e]	94.3[f]	.29
Starch	100.0	100.0	100.0	100.0	100.0	100.0	.00
Energy	74.3[b]	75.0[b]	77.4[c]	79.0[d]	81.3[e]	95.2[f]	.40
Crude protein	80.0[b]	81.6[c]	83.3[d]	84.6[de]	85.6[e]	91.9[f]	.51
Amino acids							
Indispensable							
Arginine	90.2[b]	90.9[b]	92.0[c]	92.5[c]	92.5[c]	95.9[d]	.31
Histidine	88.3[a]	88.7[b]	90.4[c]	91.5[c]	91.4[c]	95.4[d]	.38
Isoleucine	76.9[b]	78.2[b]	80.7[c]	82.5[c]	82.9[c]	89.7[d]	.67
Leucine	81.1[b]	82.2[b]	84.0[c]	85.6[d]	86.0[d]	91.7[e]	.50
Lysine	78.2[b]	80.3[b]	82.9[c]	84.5[c]	84.7[c]	93.1[d]	.81
Methionine	75.6[b]	75.1[b]	77.9[bc]	79.8[cd]	82.3[d]	88.5[e]	1.04
Phenylalanine	81.5[b]	83.2[c]	84.4[c]	86.0[d]	86.7[d]	92.0[e]	.47
Threonine	75.1[b]	75.8[b]	79.3[c]	80.6[c]	81.3[c]	89.1[d]	.89
Valine	78.1[b]	79.1[b]	81.6[c]	83.2[c]	83.4[c]	89.6[d]	.69
Dispensable							
Alanine	77.0[b]	78.3[b]	80.6[c]	81.7[c]	81.8[c]	89.1[d]	.73
Aspartic acid	79.7[b]	81.3[b]	83.1[c]	84.3[c]	84.6[c]	92.2[d]	.62
Cysteine	83.8[b]	83.1[b]	82.8[b]	86.5[c]	87.8[c]	90.1[d]	.71
Glutamic acid	89.1[b]	90.1[bc]	91.0[c]	92.2[d]	92.9[d]	94.9[e]	.38
Glycine	80.3[b]	81.2[b]	83.7[c]	84.5[c]	85.1[c]	91.1[d]	.60
Serine	83.9[b]	84.3[b]	86.0[c]	87.4[d]	87.7[d]	93.4[e]	.40
Tyrosine	74.4[b]	76.3[b]	78.9[c]	80.3[c]	81.1[c]	86.3[d]	.74

[a] Standard error of the mean

[b,c,d,e,f] Means in the same row with different superscript letters differ ($P < .05$)

Table 4-6. Disappearance (percentage units) of dry matter, starch, energy, crude protein, and amino acids in the large intestine of pigs fed the experimental diets

Item	Diets					
	A	B	C	D	E	F
Dry matter	17.9	18.6	17.8	16.4	17.1	12.3
Starch	1.9	2.4	2.1	1.8	1.3	0.0
Energy	14.1	14.8	14.4	13.4	14.8	10.5
Crude protein	9.5	9.9	10.9	9.4	10.8	10.1
Amino acids						
Indispensable						
Arginine	6.8	5.7	6.1	5.3	6.4	5.0
Histidine	8.7	7.9	8.7	7.6	8.4	8.2
Isoleucine	4.0	1.4	2.7	1.9	3.2	6.9
Leucine	6.9	4.7	5.5	4.4	5.6	8.1
Lysine	3.7	4.1	5.7	6.1	8.1	6.9
Methionine	-3.8[a]	-6.7	-3.3	-3.4	-.5	1.3
Phenylalanine	6.4	4.0	5.0	4.0	4.9	7.9
Threonine	13.0	10.1	11.1	9.3	10.6	16.1
Valine	7.4	4.2	5.7	4.6	6.0	10.3
Dispensable						
Alanine	7.5	4.3	5.2	4.2	5.8	5.0
Aspartic acid	10.2	6.9	7.7	6.5	7.9	10.8
Cysteine	18.2	16.5	19.0	14.2	19.9	20.2
Glutamic acid	6.8	6.0	4.8	4.6	5.3	7.9
Glycine	16.9	14.9	15.6	12.1	19.9	15.1
Serine	12.4	9.4	10.5	8.8	10.0	11.0
Tyrosine	8.3	8.0	9.8	6.4	10.2	10.8

[a] Minus sign indicates net synthesis in the large intestine.

Table 4-7. Disappearance (grams/kilogram of dry matter intake) of dry matter, starch, energy, crude protein, and amino acids in the large intestine of pigs fed the experimental diets

Item	Diets					
	A	B	C	D	E	F
Dry matter	178.7	185.9	178.1	164.1	171.4	122.7
Starch	1.7	2.2	1.9	2.5	2.4	.0
Energy (kcal/kg)	630.9	673.3	653.7	606.8	667.1	459.0
Crude protein	17.9	18.9	20.5	17.7	18.4	18.9
Amino acids						
Indispensable						
Arginine	.82	.69	.73	.62	2.30	.63
Histidine	.42	.38	.41	.35	.98	.37
Isoleucine	.29	.10	.19	.13	1.14	.57
Leucine	.84	.58	.67	.54	2.26	1.09
Lysine	.32	.36	.49	.51	1.62	.74
Methionine	-.11[a]	-.19	-.10	-.10	.34	.03
Phenylalanine	.51	.33	.40	.32	1.48	.69
Threonine	.82	.64	.70	.58	1.33	1.10
Valine	.65	.37	.49	.39	1.55	.90
Dispensable						
Alanine	.75	.43	.50	.40	1.62	0.46
Aspartic acid	1.66	1.14	1.25	1.04	3.10	2.17
Cysteine	.69	.63	.65	.53	1.05	.54
Glutamic acid	2.30	2.02	1.61	1.62	7.17	2.54
Glycine	1.43	1.26	1.29	.98	2.00	1.12
Serine	.98	.74	.82	.69	1.75	.93
Tyrosine	.29	.28	.34	.22	.68	.42

[a] Minus sign indicates net synthesis in the large intestine.

Table 4-8. Apparent ileal digestibilities (%) of starch, crude protein and amino acids in the wheat fractions

Item	Wheat fractions[f]					SEM[a]
	A	B	C	D	E	
Starch	98.1	97.6	97.9	98.2	98.4	0.25
Crude protein	59.2[b]	61.4[bc]	62.9[bc]	68.6[c]	67.6[c]	1.88
Amino acids						
Indispensable						
Arginine	75.5[b]	79.0[c]	80.4[c]	82.9[c]	80.2[c]	1.09
Histidine	72.3[b]	74.8[bc]	76.3[bc]	80.6[c]	78.5[bc]	1.58
Isoleucine	59.1[b]	68.5[c]	71.3[cd]	77.6[d]	75.6[d]	1.87
Leucine	62.8[b]	70.1[c]	72.3[cd]	78.4[d]	76.7[d]	1.67
Lysine	54.7	59.3	62.0	64.1	57.2	2.95
Methionine	72.2[b]	77.8[bc]	76.8[bc]	80.3[c]	79.3[bc]	1.47
Phenylalanine	63.7[b]	73.0[c]	73.3[c]	79.5[d]	79.0[d]	1.39
Threonine	48.9[b]	57.2[bc]	62.4[c]	69.2[c]	67.8[c]	3.00
Valine	62.0[b]	70.4[c]	72.4[cd]	77.9[c]	75.5[cd]	1.83
Dispensable						
Alanine	56.1[b]	64.8[c]	67.4[c]	71.0[c]	67.7[c]	2.06
Aspartic acid	50.4[b]	63.0[c]	65.9[c]	71.6[c]	68.0[c]	2.75
Cysteine	63.2[b]	64.7[b]	60.4[b]	73.6[c]	66.7[bc]	2.42
Glutamic acid	77.9[b]	81.3[bc]	85.4[cd]	88.2[d]	88.1[d]	1.49
Glycine	53.5[b]	58.6[bc]	61.9[bcd]	69.3[d]	64.9[cd]	2.53
Serine	58.5[b]	65.9[c]	67.1[c]	74.2[c]	72.3[c]	2.21
Tyrosine	53.4[b]	58.6[bc]	60.5[bc]	71.7[c]	64.8[bc]	3.99

[a] Standard error of the mean

[b,c,d,e] Means in the same row with different superscript letters differ ($P < .05$)

[f] Wheat fraction A: 70% WS and 30% WB; B: 85% WS and 15% WB; C: 100% WS; D: 85% WS and 15% WF; E: 70% WS and 30% WF.

Table 4-9. The linear relationships[a] between apparent ileal digestibilities of crude protein and amino acids and neutral-detergent fiber content in the wheat fractions

Item	Regression equations	r^2	P^b	P^c
Crude protein	$Y^d = 87.7 - .62X^e$	-.65	.0001	.019
Amino acids				
Indispensable				
Arginine	Y = 89.1 - .25X	-.42	.0001	.078
Histidine	Y = 93.1 - .44X	-.55	.0001	.021
Isoleucine	Y = 106.4 - .95X	-.61	.0001	.002
Leucine	Y = 103.8 - .83X	-.68	.0001	.001
Lysine	Y = 61.7 - .06X	-.05	.0005	.868
Methionine	Y = 85.1 - .51X	-.48	.0001	.138
Phenylalanine	Y = 106.9 - .87X	-.72	.0001	.000
Threonine	Y = 105.0 - 1.15X	-.64	.0001	.004
Valine	Y = 100.5 - .76X	-.61	.0001	.005
Dispensable				
Alanine	Y = 87.1 - .57X	-.48	.0001	.040
Aspartic acid	Y = 98.1 - .90X	-.54	.0001	.013
Cysteine	Y = 61.7 - .06X	-.40	.0001	.868
Glutamic acid	Y = 113.1 - 1.45X	-.61	.0001	.005
Glycine	Y = 90.9 - .77X	-.56	.0001	.029
Serine	Y = 100.2 - .86X	-.64	.0001	.002
Tyrosine	Y = 94.3 - .86X	-.46	.0001	.095

[a] NDF contents in the wheat fractions ranged from 29.5 to 42.3%.

[b] The probabilities of significance for the intercepts of the regression equations.

[c] The probabilities of significance for the slopes of the regression equations.

[d] Y represents digestibility coefficient (%).

[e] X represents NDF content (%).

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CHAPTER 5

ILEAL DIGESTIBILITIES OF NEUTRAL-DETERGENT FIBER, CRUDE PROTEIN AND AMINO ACIDS ASSOCIATED WITH NEUTRAL-DETERGENT FIBER IN WHEAT FRACTIONS FED TO GROWING PIGS

A. Introduction

Wheat by-products are produced when wheat is processed into flour for human consumption. Wheat bran and shorts are often used as ingredients in diets for swine. Wheat shorts comprise the layer of the wheat kernel just inside the outer bran covering endosperm and bran particles.

As was discussed in Chapter 4, the nutritive value of wheat shorts is quite variable due to different proportions of endosperm and bran particles. Part of the variation in amino acid digestibility was related to differences in the content of neutral-detergent fiber (NDF). Recent studies by Schulze et al. (1994) and Lenis et al. (1996) show that the NDF fraction is associated with significant amounts of amino acids. Amino acids associated with the NDF fraction are likely to be of low digestibility because the digestive enzymes have limited access to the cell contents as was postulated by Shah et al. (1982) and Bjergegaard et al. (1991). There is no information in the literature on the utilization of amino acids associated with the NDF fraction, which is relatively high in wheat shorts.

The objectives of these studies were to determine the ileal digestibilities of crude protein (CP) and amino acids associated with the NDF fraction from different samples of

wheat shorts (hereafter referred to as wheat fractions) and to establish a relationship between the digestibilities of CP and amino acids and CP associated with NDF in the wheat fractions.

B. Experimental Procedures

Animals and diets

A detailed description of the experimental procedures and formulation and composition of the experimental diets was presented previously (Chapter 4). Six barrows, average initial body weight (BW) 37.2 kg, were fitted with a simple T-cannula at the distal ileum and fed six diets according to a 6 × 6 Latin square design. The barrows were fed twice daily, equal amounts each meal, at 0800 and 1600. During the first experimental period, the daily dietary allowance was provided at a rate of 5% (wt/wt) of the average BW determined at the initiation of the experiment. Thereafter, the daily dietary allowance was increased by 100 g at each following experimental period.

The six diets contained 17% CP (%N × 6.25, as-fed basis). Diets A, B, C, D and E contained 17.53% soybean meal (SBM), which contributed 50% CP to these diets. The wheat fractions contained wheat shorts (WS), wheat bran (WB) or wheat flour (WF) alone or in combination, which contributed the remaining 50% of CP to these diets. Diet F contained 35.05% soybean meal, which was the sole source of dietary CP. The proportions of WS, WB and WF in the wheat fractions were 70% WS and 30% WB in diet A, 85% WS and 15% WB in diet B, 100% WS in diet C, 85% WS and 15% WF in diet D and 70% WS and 30% WF in diet E. Canola oil was included at a level of 3% and dextrose at a level of 10%. Vitamins

and minerals were supplemented according to NRC (1988) standards. Each experimental period lasted 12 d. Ileal digesta were collected for a total of 24 h; from 0800 to 1000 on d 10 and at 2-h intervals thereafter until 0600 on d 11 and from 1800 to 2000 on d 11 and at 2-h intervals thereafter until 1600 on d 12. The average BW of the barrows at the conclusion of the experiment was 90.3 kg.

The experimental proposal, surgical procedures, and procedures for care and treatment of the barrows were reviewed and approved by the Faculty of Agriculture, Forestry and Home Economics Animal Care Committee of the University of Alberta. The barrows used in this experiment were cared for in accordance with the guidelines established by CCAC (1993).

Chemical and statistical analysis

Samples of ingredients, diets and digesta were prepared for analyses as described previously as well as the methods for analyses (Chapter 4).

The preparation of the neutral-detergent solution and the procedure for isolating NDF were adapted from Goering and Van Soest (1970). Approximately 1 g of the dietary ingredients or ileal digesta was weighed into a 600 mL beaker. One hundred mL NDF solution and 100 μL α-amylase (Sigma Chemical Company, St. Louis, MO; EC 3.2.1.1) were added to the beaker and kept overnight at room temperature. The beaker was heated to boiling on a refluxing apparatus for 60 min. The solution was then filtered into a glass crucible on a filter apparatus. The filtered material was washed several times with hot water to remove the NDF solution. Thereafter, the residual material was washed with acetone several times. The crucible was dried at 110 °C overnight and weighed at 110 °C. The weight

was recorded and NDF was collected from the crucible. Then, the crucible was ashed at 500 °C overnight and placed into an oven at 110 °C for equilibration and weighed at the same temperature. The total NDF content was calculated by subtracting the weight of the ashed crucible from the weight of dried crucible containing the residual. The NDF was isolated from the sample in quadruplicate, collected and pooled. Analyses for CP and amino acids associated with NDF were performed as described in Chapter 4.

The ileal digestibility values of NDF and CP and amino acids associated with NDF in the experimental diets were subjected to statistical analysis using the General Linear Model Procedure (GLM) of SAS (1990). Means of dietary treatments, experimental periods and barrows were compared using the Student-Newman Keul's multiple range test. Pearson partial correlation analyses were conducted to determine the relationship between the apparent ileal digestibilities of CP and amino acids in the wheat fractions and the content of CP associated with NDF in the wheat fractions. Variation contributed by barrows and periods were removed and correlation coefficients were computed from the Error SS&CP (df = 19) using the repeated measurement option of GLM of SAS (1990). The regression equations were developed using the Regression Procedure of SAS (1990).

C. Results and Discussion

The amino acid content of WB, WS and SBM was previously presented (Chapter 4). The contents of CP and amino acids in NDF and the contribution to the total CP and amino acids in SBM, WB and WS are presented in Table 5-1. The contents of CP and most of the amino acids were highest in NDF isolated from WB. In all ingredients, the highest

contributions were observed for aspartic and glutamic acid. As expected, reflecting the content of NDF, the contribution of CP and amino acids associated with NDF to total CP and amino acids was highest in WB and lowest in SBM. Of the indispensable amino acids, these values ranged from 16.7 (histidine) to 19.7% (valine) in WB. In WS, these values ranged from 12.2 to 15.1%, also for histidine and valine, respectively.

The CP and amino acid content and the contribution of CP and amino acids associated with NDF to the total CP and amino acids in the wheat fractions are presented in Table 5-2. The amino acid content of the wheat fractions was not previously presented. The contribution of CP and amino acids gradually decreased from wheat fraction A to E, reflecting a decrease in NDF content from 42.3 to 29.5%. Of the indispensable amino acids in wheat fraction A, the contributions ranged from 12.9 (histidine) to 15.9% (valine). In wheat fraction E, these values ranged from 9.0 (histidine) to 11.3% (lysine). In all wheat fractions, of the dispensable amino acids, the highest and lowest contributions were observed for glycine and glutamic acid, respectively.

The contributions of CP and amino acids associated with NDF to total CP and amino acids in ileal digesta of pigs fed the experimental diets are presented in Table 5-3. These values should reflect those of the respective wheat fractions in the experimental diets as the contribution by SBM to the total NDF content in the diet is very small (4.5 to 6.1%). These studies show that a considerable proportion of amino acids in ileal digesta are associated with NDF. Of the indispensable amino acids, the values ranged from 8.6% for lysine in pigs fed diet E to 21.9% for methionine in pigs fed diet B. For all diets, the contributions of lysine and methionine were lowest and highest, respectively. It is of interest to note that the contribution

of CP associated with NDF to total CP in ileal digesta was lower than the contributions of amino acids associated with NDF to total amino acids in ileal digesta. This results from the fact that a considerable proportion of CP in ileal digesta is present in the form of non-amino acid nitrogen. Sauer (1976) and Landin (1992), in studies with pigs fed protein-free diets, reported that 27.8 to 44.5 and 41.7% of the nitrogen was present in the form of non-amino acid nitrogen, respectively. Non-amino acid nitrogen includes nitrogen from amino sugars (glucosamine and galactosamine) (Landin, 1992) and likely ammonia and urea (Mosenthin, 1987).

The apparent ileal digestibilities of NDF are presented in Table 5-4. Although the apparent ileal digestibilities of NDF ranged from 11.8% in diet E to 17.1% in diet C, these differences were not significant ($P > .05$). Schulze et al. (1994) also observed no differences ($P > .05$) in the ileal digestibilities of NDF in studies with pigs fed diets that included 6, 12 and 18% purified NDF from wheat bran. The ileal digestibilities of NDF ranged between 16.2 and 18%. Pigs do not produce enzymes that digest NDF. Therefore, the disappearance of NDF in the small intestine must result from bacterial fermentation. Disappearance of NDF in the small intestine was also reported by Buraczewska et al. (1988); the values ranged from 10 to 32% depending on the source of NDF. They postulated that the hemicellulose fraction of NDF is most prone to bacterial fermentation in the small intestine. The presence of significant bacterial fermentation in the small intestine (as opposed to the large intestine) has often been overlooked and was therefore examined in studies presented in Chapter 6.

There were usually no differences ($P > .05$) in the ileal digestibilities of amino acids associated with NDF between the experimental diets (Table 5-4). The digestibilities of amino

acids associated with NDF were low. The average digestibilities of the indispensable amino acids (over all diets) ranged from 48.2% (isoleucine) to 66.8% (arginine). Furthermore, it should be pointed out that these digestibility values represent true rather than apparent values. The apparent values will be lower as dietary fiber per sé may affect the amount of endogenous amino acids in ileal digesta and decrease nutrient absorption. As was pointed out previously, the level and source of dietary fiber are two of the most, in addition to the presence of antinutritional compounds, important factors that affect the levels of endogenous amino acids in ileal digesta (Sauer and Ozimek, 1986). The NDF fraction comprises a heterogenous mixture of structural (cellulose, hemicellulose, and pectins) and non-structural polysaccharides and lignin (Goering and Van Soest, 1970). The inclusion of water-insoluble fiber increased the sloughing of intestinal mucosal cells and mucus production (Schneeman et al., 1982). However, there is still controversy as to whether fiber affects the production of mucus (Leterme et al., 1992). This aspect was investigated further in Chapter 6. Several studies have shown an increase in the level of endogenous protein and amino acids in digesta collected from the distal ileum with the inclusion of water-insoluble fiber sources in a protein-free diet (Sauer et al., 1977; Taverner et al., 1981; De Lange et al., 1989; Furuya and Kaji, 1992; Leterme et al., 1992). The increases were not always significant but there was always a trend in the other studies. Li et al. (1994) pointed out that amino acid losses, resulting from the dietary inclusion of water-insoluble fiber, may become quantitatively important only when a certain level (threshold level) is exceeded. On the other hand, the dietary inclusion of water-soluble fiber, due to its gelling and viscosity properties, will decrease digestion and absorption of nutrients by reducing the mixing of intestinal contents,

blocking enzyme-substrate interaction, and by forming an unstirred layer, thereby creating a physical barrier to nutrient absorption (Johnson and Gee, 1981). Studies with growing pigs showed that the inclusion of 7.5% pectin in a 18% CP corn starch-based SBM diet decreased ($P < .05$) the ileal amino acid digestibilities (Mosenthin et al., 1994).

In addition to increasing the sloughing of intestinal cells, and perhaps mucus production and decreasing nutrient absorption, the inclusion of dietary fiber may also increase the secretion of protein from pancreatic juice and thereby enhance the endogenous amino acid losses. Langlois et al. (1987) reported an increase ($P < .05$) in pancreatic protein output (19.7 vs 14.5 g/d) when 40% wheat bran was included in a wheat-based diet. Zebrowska (1985) also reported an increase in pancreatic protein output (19.0 vs 17.9 g/d) when 44% wheat bran was included in a wheat-based diet. The difference, however, was not significant at $P < .05$. Furthermore, studies by Mosenthin and Sauer (1991) and Mosenthin et al. (1994) showed no effect of the dietary inclusion of fiber on pancreatic protein output. Even in the case there is an increase in pancreatic protein output with the inclusion of dietary fiber, this will only have a minor effect on the recovery of endogenous protein at the distal ileum. According to Souffrant (1991), approximately 90% of pancreatic protein is recycled in the small intestine.

The content of CP (% N × 6.25) associated with NDF in the wheat fractions ranged from 1.16 to 1.84%. Simple linear relationships were established between the apparent ileal digestibilities of CP and amino acids and the content (%) of CP associated with NDF in the wheat fractions (Table 5-5). With the exception of lysine, methionine, cysteine and tyrosine, there were significant ($P < .05$) negative correlations between the two factors. The

correlations were almost significant for methionine ($P < .056$) and tyrosine ($P < .069$). The poor correlation of the apparent ileal digestibility of lysine with the content of CP associated with NDF in the wheat fractions may be attributed to the fact that there were no differences ($P > .05$) in lysine digestibilities between the wheat fractions (Chapter 4). The correlations between apparent ileal amino acid digestibilities and the content of CP associated with NDF were higher than those between apparent ileal amino acid digestibilities and NDF content in the wheat fractions, the latter were presented in Chapter 4.

In conclusion, a considerable proportion of CP and amino acids are associated with NDF in the wheat fractions and are of low digestibility. Furthermore, there were negative correlations between the apparent ileal digestibilities of most of the amino acids and the content of CP associated with the NDF fraction.

Table 5-1. Content[a] (%) of crude protein and amino acids in neutral-detergent fiber and the contribution (%) to the total crude protein and amino acid content in soybean meal, wheat bran and wheat shorts

Item	Ingredients					
	Soybean meal		Wheat bran		Wheat shorts	
	Cont.[b]	Contr.[c]	Cont.	Contr.	Cont.	Contr.
Crude protein	5.01	.65	5.37	14.00	4.16	9.85
Amino acids						
Indispensable						
Arginine	.24	.47	.47	18.59	.35	13.60
Histidine	.13	.69	.17	16.67	.13	12.17
Isoleucine	.24	.70	.22	17.72	.19	14.23
Leucine	.37	.67	.40	17.51	.33	13.46
Lysine	.33	.72	.25	19.11	.20	13.51
Methionine	.10	.91	.12	19.33	.09	13.24
Phenylalanine	.24	.66	.25	16.83	.21	13.52
Threonine	.21	.74	.22	18.37	.19	14.77
Valine	.31	.86	.35	19.73	.29	15.12
Dispensable						
Alanine	.26	.68	.37	17.38	.31	13.44
Aspartic acid	.46	.56	.48	18.66	.39	13.97
Cysteine	.04	.36	.14	13.72	.12	10.99
Glutamic acid	.63	.48	.71	9.67	.55	7.10
Glycine	.38	1.25	.44	22.05	.36	17.25
Serine	.24	.68	.21	14.13	.18	11.41
Tyrosine	.15	.93	.11	19.08	.10	15.26

[a] Dry matter basis.

[b] Content.

[c] Contribution.

Table 5-2. Content[a] (%) of crude protein and amino acids and the contribution (%) of crude protein and amino acids associated with the neutral-detergent fiber fraction to the total crude protein and amino acid content in the wheat fractions

Item	Wheat fractions[b]									
	A		B		C		D		E	
	Cont.[c]	Contr.[d]	Cont.	Contr.	Cont.	Contr.	Cont.	Contr.	Cont.	Contr.
Crude protein	1.84	10.60	1.75	10.04	1.65	9.49	1.40	8.19	1.16	6.85
Amino acids										
Indispensable										
Arginine	.16	14.56	.15	13.84	.14	13.10	.12	12.04	.10	10.80
Histidine	.06	12.85	.05	12.43	.05	11.72	.04	10.44	.04	9.03
Isoleucine	.08	14.71	.08	14.21	.08	13.71	.06	11.65	.05	9.59
Leucine	.14	14.00	.14	13.55	.13	12.96	.11	11.02	.09	9.07
Lysine	.09	14.59	.08	13.68	.08	13.01	.07	12.26	.06	11.34
Methionine	.04	14.41	.04	13.58	.04	12.75	.03	11.24	.02	9.26
Phenylalanine	.09	13.89	.09	13.56	.08	13.02	.07	10.73	.06	8.70
Threonine	.08	15.26	.08	14.74	.08	14.22	.06	12.56	.05	10.77
Valine	.13	15.86	.12	15.21	.12	14.56	.10	12.70	.08	10.88
Dispensable										
Alanine	.13	14.04	.13	13.49	.12	12.95	.10	11.88	.09	10.50
Aspartic acid	.17	14.76	.16	14.11	.15	13.46	.13	12.29	.11	10.94
Cysteine	.05	11.38	.05	10.98	.05	10.58	.04	9.20	.03	7.75
Glutamic acid	.24	7.54	.23	7.19	.22	6.84	.19	5.31	.15	4.03
Glycine	.16	17.96	.15	17.19	.14	16.61	.12	14.99	.10	13.16
Serine	.08	11.64	.07	11.40	.07	10.99	.06	9.34	.05	7.69
Tyrosine	.04	15.50	.04	15.09	.04	14.69	.03	12.49	.03	10.29

[a] Dry matter basis.
[b] Wheat fraction A: 70% WS and 30% WB; B: 85% WS and 15% WB; C: 100% WS; D: 85% WS and 15% WF; E: 70% WS and 30% WF.
[c] Content.
[d] Contribution.

Table 5-3. Contribution (%) of crude protein and amino acids associated with neutral-detergent fiber to the total crude protein and amino acids in the ileal digesta

Item	Diets				
	A	B	C	D	E
Crude protein	5.71	5.27	5.79	5.49	5.49
Amino acids					
Indispensable					
Arginine	14.46	15.20	16.04	16.43	14.20
Histidine	14.40	14.01	15.72	15.94	14.10
Isoleucine	12.43	14.12	14.64	14.89	13.66
Leucine	12.71	14.12	14.53	14.89	13.45
Lysine	8.91	8.75	10.01	9.64	8.64
Methionine	19.56	21.85	19.47	19.85	19.30
Phenylalanine	13.36	14.92	15.15	15.48	14.02
Threonine	9.92	10.43	11.19	10.90	10.70
Valine	13.47	15.35	15.79	16.00	14.61
Dispensable					
Alanine	12.36	14.01	14.54	14.71	13.61
Aspartic acid	10.27	11.48	11.89	11.94	11.18
Cysteine	11.22	11.29	10.88	11.80	10.36
Glutamic acid	9.56	10.44	11.86	11.08	10.21
Glycine	15.84	16.94	17.37	18.04	16.73
Serine	10.46	11.23	11.63	11.48	10.58
Tyrosine	12.35	12.01	12.40	13.10	11.79

Table 5-4. The ileal digestibilities of neutral-detergent fiber and crude protein and amino acids associated with neutral-detergent fiber in the experimental diets

Item	Diets					SEM[a]
	A	B	C	D	E	
NDF	13.9	13.0	17.1	15.8	11.8	2.04
Crude protein	71.0	72.8	69.7	70.8	66.3	1.66
Amino acids						
Indispensable						
Arginine	68.4	68.5	67.0	66.1	63.9	1.25
Histidine	58.9	60.7	56.9	56.3	53.6	1.75
Isoleucine	49.9	50.0	49.3	48.8	42.9	1.93
Leucine	52.5	51.9	51.6	50.3	45.4	1.68
Lysine	63.2[b]	64.6[b]	60.2[bc]	60.0[bc]	56.3[c]	1.63
Methionine	51.8	49.8	51.2	50.0	44.0	2.18
Phenylalanine	51.3	51.0	51.1	50.1	45.1	1.79
Threonine	50.6	51.8	50.8	51.7	43.9	2.04
Valine	53.6	53.1	52.7	51.8	46.6	1.71
Dispensable						
Alanine	52.4	52.6	52.3	51.4	46.0	1.68
Aspartic acid	49.6	50.5	49.2	49.2	43.1	1.83
Cysteine	50.9	50.3	51.8	49.9	45.2	1.61
Glutamic acid	43.4	48.3	47.4	45.8	38.2	1.55
Glycine	47.2	46.8	46.9	46.9	40.7	1.83
Serine	49.4	50.6	49.5	50.2	44.5	1.98
Tyrosine	53.4[b]	58.6[bc]	60.5[bc]	71.7[c]	64.8[bc]	2.51

[a] Standard error of the mean.

[b,c] Means in the same row with different superscript letters differ ($P < .05$).

Table 5-5. The linear relationships between the apparent ileal digestibilities of crude protein and amino acids and crude protein[a] associated with neutral-detergent fiber in the wheat fractions

Item	Regression equations	r^2	P^b	P^c
Crude protein	$Y^d = 85.4 - 12.89X^e$	-.69	.0001	.012
Amino acids				
Indispensable				
Arginine	Y = 91.3 - 9.88X	-.57	.0001	.032
Histidine	Y = 92.1 - 9.38X	-.62	.0001	.010
Isoleucine	Y = 104.6 - 20.60X	-.74	.0001	.000
Leucine	Y = 102.0 - 18.01X	-.75	.0001	.000
Lysine	Y = 64.2 - 2.84X	-.10	.0001	.677
Methionine	Y = 89.4 - 7.11X	-.39	.0001	.056
Phenylalanine	Y = 104.7 - 18.64X	-.77	.0001	.000
Threonine	Y = 102.4 - 24.84X	-.71	.0001	.001
Valine	Y = 99.3 - 16.67X	-.68	.0001	.001
Dispensable				
Alanine	Y = 87.4 - 13.22X	-.60	.0001	.013
Aspartic acid	Y = 97.6 - 20.35X	-.64	.0001	.004
Cysteine	Y = 82.5 - 10.09X	-.30	.0001	.163
Glutamic acid	Y = 106.7 - 13.54X	-.73	.0001	.000
Glycine	Y = 89.5 - 16.71X	-.63	.0001	.014
Serine	Y = 98.0 - 18.29X	-.69	.0001	.001
Tyrosine	Y = 92.4 - 18.43X	-.49	.0001	.069

[a] Content of CP associated with NDF in the wheat fractions ranged from 1.16 to 1.84%.

[b] The probabilities of significance for the intercepts of the regression equations.

[c] The probabilities of significance for the slopes of the regression equations.

[d] Y represents digestibility coefficient (%).

[e] X represents content (%) of CP associated with NDF.

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CHAPTER 6

THE RECOVERY OF MUCIN IN ILEAL DIGESTA AND BACTERIAL NITROGEN IN ILEAL DIGESTA AND FECES OF GROWING PIGS FED DIETS DIFFERING IN THE CONTENT OF NEUTRAL-DETERGENT FIBER

A. Introduction

The apparent ileal digestibilities of amino acids in a diet refer to the difference between the amount of each amino acid consumed and recovered in digesta collected from the distal ileum. The digesta that is collected is a mixture of variable proportions of material from exogenous, endogenous and bacterial origin. For the determination of true amino acid digestibilities it is therefore important to quantify the amount of amino acids that originate from endogenous and bacterial protein.

Fiber-induced alterations in the production of mucin in the gastrointestinal tract may have important functional properties with respect to nutrient absorption. Evidence suggests that once in the intestinal lumen, little degradation occurs prior to the large intestine, where it is degraded by the microflora (Hoskins, 1984). Therefore, amino acids derived from mucin may represent a considerable proportion of endogenous amino acids recovered in digesta collected from the distal ileum. In addition, mucin has also been implicated as an important component of the unstirred water layer (Smithson et al., 1981), and has been suggested to act as a surface barrier to the intestinal absorption of nutrients (Vahouny et al., 1985; Smithson et al., 1981).

A number of microbiological studies with pigs have shown the presence of bacteria throughout the entire digestive tract. Many studies have been carried out to determine the activity of the microflora in the large intestine. The activity of the microflora in the small intestine has only been investigated in a few studies. Bacteria can directly use amino acids from exogenous and endogenous origin in addition to synthesizing their own amino acids from non-protein nitrogen (mainly from urea) (Mosenthin, 1987). For the accurate determination of true amino acid digestibility, it is therefore important to estimate the content of bacterial protein in ileal digesta as well.

The objectives of this study were to determine the recovery of mucin in ileal digesta and bacterial nitrogen in ileal digesta and feces of pigs fed diets differing in neutral-detergent fiber (NDF) content.

B. Experimental Procedures

Animals and diets

A detailed description of the experimental procedures and formulation and composition of the experimental diets was presented previously (Chapter 4). Six barrows, average initial body weight (BW) 37.2 kg, were fitted with a simple T-cannula at the distal ileum and fed six diets according to a 6 × 6 Latin square design. The barrows were fed twice daily, equal amounts each meal, at 0800 and 1600. During the first experimental period, the daily dietary allowance was provided at a rate of 5% (wt/wt) of the average BW determined at the initiation of the experiment. Thereafter, the daily dietary allowance was increased by 100 g at each following experimental period.

The six diets contained 17% crude protein (CP) (%N × 6.25, as-fed basis). Diets A, B, C, D and E contained 17.53% soybean meal (SBM), which contributed 50% CP to these diets. The wheat fractions contained wheat shorts (WS), wheat bran (WB) or wheat flour (WF) alone or in combination, which contributed the remaining 50% CP to these diets. Diet F contained 35.05% soybean meal, which was the sole source of dietary CP. The proportions (%) of WS, WB and WF in the wheat fractions were 70% WS and 30% WB in diet A, 85% WS and 15% WB in diet B, 100% WS in diet C, 85% WS and 15% WF in diet D and 70% WS and 30% WF in diet E. Canola oil was included at a level of 3% and dextrose at a level of 10%. Vitamins and minerals were supplemented according to NRC (1988) standards. Each experimental period lasted 12 d. Feces were collected for 48 h from 0800 on d 8 to 0800 on d 10. Ileal digesta were collected for a total of 24 h; from 0800 to 1000 on d 10 and at 2-h intervals thereafter until 0600 on d 11 and from 1800 to 2000 on d 11 and at 2-h intervals thereafter until 1600 on d 12. The average BW of the barrows at the conclusion of the experiment was 90.3 kg.

The experimental proposal, surgical procedures, and procedures for care and treatment of the barrows were reviewed and approved by the Faculty of Agriculture and Forestry Animal Care Committee of the University of Alberta. The barrows used in this experiment were cared for in accordance with the guidelines established by CCAC (1984).

Chemical analysis

Crude mucin (CM) was isolated from digesta according to modified procedures described by Allen (1981), Miller and Hoskins (1981) and Lien (1995). Approximately 3 g of freeze-dried digesta was weighed into a 50 mL polystyrene test tube and 25 mL of 0.15M

NaCl containing .02 M sodium azide, maintained at 4 °C, was added and samples were homogenized for 1 min using a Polytron Homogenizer (Kinematica, Kriens, Switzerland). Homogenized samples were then immediately centrifuged for 30 min at 12,000 × g at 4 °C. and the aqueous layer decanted into a second 50 mL polystyrene test tube. The aqueous layer was centrifuged again at 12,000 × g for 30 min to ensure the complete removal of insoluble material. Ten mL of the aqueous fraction was pipetted into a pre-weighed 50 mL polystyrene tube, cooled in an ice-bath, and ice cold ethanol added to a final concentration of 60% (v/v). The samples were allowed to precipitate overnight at -20 °C.

The following day samples were centrifuged at 1,400 × g for 10 min at 4 °C and the precipitate recovered by decanting the supernatant. The pellet was resolubilized in 15 mL of .15 M NaCl and cooled in an ice-bath. Pre-chilled ethanol was added to a final concentration of 60% (v/v) and the samples were left overnight to precipitate as before. The CM precipitate was recovered as described previously. The precipitate was resolubilized in 10 mL of water and freeze-dried.

Carbohydrates were analyzed as their alditol acetates according to procedures adapted from Blakeney et al. (1983) and Kraus et al. (1990). Approximately 50 mg of CM was treated with 12 M sulfuric acid (1.5 mL) for 1 h at room temperature. The solution was diluted to 3 M with 4.5 mL of water and the samples hydrolyzed for 1 h at 110 °C. Following hydrolysis, 200 µL of internal standard were added (N-methylglucamine and myo-inositol for amino sugars and neutral sugars, respectively, at 10 mg/mL of distilled water). Aliquots (1 mL) of the acid hydrolysates were cooled in an ice-bath and made basic with the addition

of 0.7 mL concentrated ammonium hydroxide. To 100 µL of this, 1 mL sodium borohydride (30 mg/mL in anhydrous dimethylsulphoxide) was added and reduction allowed to proceed for 90 min at 40 °C. Excess sodium borohydrate was decomposed with the addition of 200 µL concentrated glacial acetic acid. Following this, .2 mL 1-methylimidazole and then 2 mL acetic anhydride were added. The solution was mixed and acetylation occurred at room temperature for 10 to 15 min. Thereafter, 5 mL of water was added to decompose excess acetic anhydride and the mixture cooled to room temperature. Alditol acetates were extracted into 4 mL of dichloromethane by vigorous shaking and the upper aqueous layer removed. The dichloromethane layer was rinsed twice with 4 mL of water. Standard sugars and derivitization reagents were purchased from Sigma (Sigma Chemical, St. Louis, MO).

The samples were analyzed by gas-liquid mass chromatography (Hewlett Packard 5890 Series II Gas Chromatograph) and approximately .5 µL of derivatized sample was injected onto a DB-17 fused silica capillary column (.25 mm i.d. × 30 m; J&W Scientific, Folsom, CA). Helium was used as the carrier gas at a rate of 1.5 mL/min. Injector temperature was programmed from 60 °C to 270 °C at 150 °C/min and maintained for 20 min. Oven temperature was raised at 30 °C/min from 50 °C 190 °C, held for 3 min, then increased 5 °C/min to 270 °C and held for 5 min. The detector (Hewlett Packard 5971A Mass Selective Detector) temperature was set at 270 °C. The peak area was integrated using MS ChemStation Software (1993, Hewlett Packard G1030C).

Calculations

Regression equations were derived from the N-acetylglucosamine (GlcNAc) to N-acetylgalactosamine (GalNAc) ratios in purified gastric (Scawen and Allen, 1977) and

intestinal (Mantle and Allen, 1981; Mantle et al., 1981) mucins to calculate the contributions of gastric mucin and the GalNAc content. The formula was derived, assuming complete native (no proteolytic digestion) mucin, to estimate the range of mucin output. The relationship between the GlcNAc/GalNAc ratio and contribution of gastric mucin is described by the following regression equation:

$$\%\text{gastric mucin} = -80.23 + 183.26x - 71.19x^2 + 11.05x^3 \quad (1)$$

where x = the GlcNAc/GalNAc ratio. The GalNAc content of mucin mixtures is described by the following regression equation:

$$\%\text{GalNAc} = 32.30 - 22.74x + 8.83x^2 - 1.37x^3 \quad (2)$$

where x = the GlcNAc/GalNAc ratio. Daily output of mucin was calculated from the estimated GalNAc content and daily outputs of GalNAc in CM or ileal digesta by the following equation:

$$\text{mucin output} = \text{GalNAc}/\%\text{GalNAc} \quad (3)$$

where GalNAc = GalNAc output in g/day.

For diaminopimelic acid (DAPA) analyses, approximately .2 g of sample was weighed into a screw-capped test tube and mixed with 3 mL of 6 N HCL. The tubes were purged with nitrogen and then hydrolyzed in an oven at 110 °C for 24 h. The hydrolyzed samples were mixed with the internal standard, DL-α-aminocaprylic acid, and centrifuged at 1,500 × g for 15 min at 4°C. The supernatant of the sample was analyzed according to principles outlined by Dugan et al. (1992) using a Varian 5000 high performance liquid chromatography system with a reverse-phase column and a Varian Fluorichrom detector (Varian Canada Inc., Mississauga, ON). The DAPA were derivatized with an o-

phthaldialdehyde reagent solution. The mobile phase consisted of a binary gradient changing from a polar to a non-polar solvent with a flow rate of 1.1 mL/min. The polar solvent consisted of a water-methanol mixture (60:40, v/v) containing 0.1 M sodium acetate and 7.5mM hexadecyltrimethylammonium bromide (HTMA). The non-polar solvent consisted of a methanol-water mixture (95:5, v/v) containing 7.5 mM HTMA. Peaks were recorded and integrated using the Ezchrom™ Chromatography Data System (version 2.12; Shimadzu Scientific Instruments Inc., Columbia, MD).

The bacterial nitrogen content in ileal digesta was estimated by measuring the DAPA content in ileal digesta of the pigs fed the different experimental diets and calculated by using the value of 26.4 mg of DAPA/g of bacterial nitrogen according to Wünsche et al. (1991).

Statistical Analyses

To determine treatment effects, data were subjected to statistical analysis using the General Linear Model Procedure of SAS (1990). Means of dietary treatments were compared using the Student-Newman Keul's multiple range test.

C. Results and Discussion

Mucus, a complex secretion of the epithelial lining of the gastrointestinal tract, consists mainly of water, immunoglobulins, enzymes and mucins. Mucins are high molecular weight glycoproteins responsible for the gelling nature of mucus (La Mont, 1985). The interest in gastrointestinal synthesis and secretion of mucins is based on an increasing list of assumed functions ascribed to these intestinal surface constituents (Forstner et al., 1976; Cassidy et al., 1981). Among these functions are cytoprotection of mucosal cells, antigenic

responses, and antiviral and antibacterial activities (Cassidy et al., 1981).

The daily outputs of mucin in digesta collected from the distal ileum of pigs fed the experimental diets are presented in Table 6-1. There were no differences ($P > .05$) in the daily outputs of mucin among the diets. These results are in agreement with some but not all studies reported in the literature. Satchithanandam et al. (1996), in studies with rats fed diets containing 2 levels of soluble (psyllium) and insoluble fiber (cellulose and rice bran), found no differences ($P > .05$) in the amount of mucin produced by small intestine compared to that of rats fed the control diet. Furthermore, in rats fed wheat bran, there were no differences in morphological appearance of the jejunum, in the number of cells/villus column or in the number of goblet cells compared to rats fed the control diet (Vahouny et al., 1985). The goblet cells are derived from undifferentiated intestinal crypt cells, and during cell migration and differentiation, become clearly discernible from enterocytes, and represent about one of every eight cells on the villus column. These cells secrete mucus substances which line the mucosal surface. Jacobs and White (1983) fed rats wheat bran for 4 wk. Compared to the controls fed a fiber-free diet, there was no effect on small intestinal mucosal weight, DNA and DNA synthesis. This was in contrast to large intestinal mucosal DNA which increased by 59.1% in the cecum, by 28.3% in the proximal colon and by 35.6% in the distal colon. The aforementioned results show that the mechanisms by which dietary fiber affects intestinal mucosa will vary in different segments of the intestine.

Digestion of purified mucin with trypsin, pepsin or pronase showed the presence of two distinct regions in the glycoprotein molecule. The first one is rich in threonine, serine and proline and is glycosylated and resistant to proteolysis. The second one, which has an

amino acid composition more characteristic of a globular protein, is not glycosylated and is susceptible to proteolysis (Scawen and Allen, 1977). Lien (1995) reported that the contributions of threonine (28 to 35%), serine (13 to 16%) and proline (7 to 24%) from mucin to their respective total amounts were highest for these amino acids in endogenous protein recovered at the distal ileum. The studies by Lien (1995) explain the relatively low apparent ileal digestibilities of threonine and proline in many feedstuffs (Sauer et al., 1977 a,b; Sauer and Ozimek, 1986). Relatively low apparent ileal digestibilities for threonine were also observed in the wheat fractions which were presented in Chapter 4. Hoskins (1984) reported that the degradation of mucin occurs primarily by the microflora in the large intestine. Fermentation of mucin by the microflora also explains the relatively large disappearance of threonine and proline in the large intestine (Chapter 4; Sauer et al., 1977 a,b; Sauer and Ozimek, 1986).

The daily outputs of GlcNAc, GalNAc and GlcNAc to GalNAc ratios are also presented in Table 6-1. There were no differences in the daily outputs and ratios among the experimental diets. The GlcNAc to GalNAc ratio is 2.35 for pig gastric mucin (Scawen and Allen, 1977) and .55 for pig small intestinal mucin (Mantle and Allen, 1981). The average ratio obtained in the present experiment was .42, ranging from .40 to .45. These results show that there was no effect of dietary NDF level on the contribution of gastric and intestinal mucin to the total mucin output. The reason for the lower ratio in this study than the value reported by Mantle and Allen (1981) is not known. However, the lower ratio may indicate the lower contribution of gastric mucin to total mucin recovered at the distal ileum, which may be caused by microbial activity in the small intestine. Mucin is an important source of

nutrients for many intestinal organisms and bacteria may selectively use host polysaccharides (mucin) and dietary polysaccharides for fermentation (Rowland and Mallett, 1990).

The output of mucin in feces was not measured in this study because of considerable microbial fermentation of mucin in the large intestine (Hoskins, 1984).

Diaminopimelic acid is present in bacterial cell wall mucoprotein but is not found in plant or animal cells (Rowan et al., 1992). Its specificity to bacteria is therefore a major advantage when used as a marker, but a disadvantage is that its concentration varies among different species of bacteria (Czerkawski, 1974). The amount of bacterial protein in a digesta sample is calculated by multiplying the DAPA concentration in the digesta sample by the protein-to-DAPA ratio (26.4) found in purified bacteria collected from the digesta sample (Wünsche et al., 1991). Diaminopimelic acid could not be detected in the diets. The daily amounts of DAPA and bacterial nitrogen recovered in ileal digesta and feces are presented in Table 6-2. There were no differences ($P > .05$) in daily ileal outputs of DAPA and bacterial nitrogen between pigs fed the experimental diets. The average output of DAPA at the distal ileum was 61.79 mg/d, ranging from 48.15 to 75.62 mg/d. The average ileal output of bacterial nitrogen was 2.34 g/d, ranging from 1.82 to 2.86 g/d. The values for DAPA were similar to those reported by Schulze et al. (1994) in which pigs were fed diets containing 6.1 to 17.7% NDF purified from wheat bran. They reported values that ranged from 48.24 to 58.48 mg/d.

There was a difference ($P < .05$) in the contribution of bacterial nitrogen to total nitrogen in ileal digesta (Table 6-2). The contribution was higher ($P < .05$) when the pigs were fed diet F (34.0%) compared to the other diets, in which the contributions ranged from

14.0 to 21.7%. These values were lower than those reported by Schulze et al. (1994) which ranged from 54.4 to 64.7%. The difference between the values in this study and those of Schulze et al. (1994) may result from different cannulation techniques. Schulze et al. (1994) fitted pigs with a post-valvular T-cecal cannula whereas pigs in these studies were fitted a simple T-cannula. Digesta collected in pigs fitted with a post-valvular T-cecal cannula may be contaminated with bacteria from the large intestine. It is of interest to point out here that in human beings with an established ileostomy, the number of bacteria recovered from ileal effluent was 80 times higher than in normal ileal contents (Gorbach et al., 1967). The lower contribution of bacterial nitrogen to total nitrogen in ileal digesta when pigs were fed diets A, B, C, D, and E compared to diet F is likely due to the higher daily nitrogen flow at the distal ileum, because of the lower digestibility of dietary nitrogen and higher endogenous nitrogen losses as was discussed in Chapter 5. These results show that the effect of microbial protein on apparent ileal amino acid digestibility is greater for highly digestible than for lowly digestible protein sources.

The outputs of DAPA and bacterial nitrogen and contributions of bacterial to total nitrogen were considerably higher in feces than in ileal digesta (Table 6-2). The output of bacterial nitrogen in feces was higher ($P < .05$) when the pigs were fed diets A, B, C, D and E (6.0 to 7.5 g/d) than diet F (3.8 g/d) which resulted from a larger amount of fermentable substances entering the large intestine thereby increasing microbial activity. Carbohydrates that are not absorbed in the small intestine will increase nitrogen losses in feces and decrease nitrogen losses in urine, which results from an increase in microbial activity in the large intestine. Ammonia is a principle by-product of microbial fermentation. In the presence of

sufficient fermentable carbohydrates, ammonia will be incorporated into microbial protein rather than being absorbed from the large intestine (Misir and Sauer, 1982; Nyman and Asp, 1982). Whiting and Bezeau (1957a,b) reported that the type and amount of dietary fiber have a considerable effect on fecal nitrogen excretion in pigs whether expressed as a proportion of dry matter (DM) intake or fecal DM output. On the other hand, Mason and Palmer (1973) believed that it was not the amount but the extent of fermentation of dietary DM which resulted in the production of more bacterial cells and hence more bacterial residues appearing in feces, thus increasing the amount of fecal nitrogen. Thus, according to Mason and Palmer (1973), fiber sources that undergo extensive degradation in the large intestine of the pig will decrease the apparent digestibility of nitrogen to a larger extent than fiber sources less susceptible to microbial fermentation provided they are fed at similar levels.

The effect of fermentable polysaccharides in the large intestine on urea nitrogen disposal and nitrogen fecal excretion is quite interesting. Increased microbial protein synthesis in the large intestine shifts nitrogen elimination as urea via the kidney into the large intestine as fecal bacterial nitrogen (Misir and Sauer, 1982; Younges et al., 1995). When the diet contains more fermentable carbohydrates, nitrogen required for optimal bacterial growth must be provided by dietary proteins escaping breakdown in the small intestine, endogenous proteins (pancreatic and intestinal secretions and sloughed epithelial cells), or blood urea diffusing into the digestive contents (Mason, 1984; MacFarlane and Cummings, 1991).

There was a difference ($P < .05$) in the contribution of bacterial nitrogen to total nitrogen in feces when the pigs were fed diets A, B, C, D and E (80.2 to 87.7%) compared to diet F (92.8%) (Table 6-2). These values reflect the relative differences for these diets in

ileal digesta, the reasons which were previously explained. Sauer et al. (1991), Rowan et al. (1992) and Mosenthin et al. (1994) also reported large proportions of bacterial nitrogen in feces with values ranging from 70 to 86%. The present study shows once more that the modifying action of the microflora in the large intestine.

In conclusion, there was no effect ($P > .05$) of dietary fiber levels on the total outputs of mucin and bacterial nitrogen in digesta collected from the distal ileum. Depending on the level of fiber, the proportions of bacterial nitrogen in ileal digesta range from 14 to 34% and from 80.2 to 92.8% in feces.

Table 6-1. Outputs of mucin, N-acetylglucosamine, N-acetylgalactosamine and N-acetylglucosamine/N-acetylgalactosamine ratios in ileal digesta of pigs fed the experimental diets

Item	Diets						SEM[a]
	A	B	C	D	E	F	
Mucin, g/d	12.64	11.43	11.93	10.90	11.13	14.26	.327
GlcNAc, g/d	1.12	.99	1.03	.94	.99	1.23	.027
GalNAc, g/d	2.55	2.42	2.53	2.29	2.20	3.04	.056
GlcNAc/GalNAc ratio	.44	.41	.41	.41	.45	.40	.022

[a] Standard error of the mean

Table 6-2. Outputs of diaminopimelic acid and bacterial nitrogen in ileal digesta and feces and the contribution of bacterial to total nitrogen of pigs fed the experimental diets

Item	Diets						SEM[a]
	A	B	C	D	E	F	
Ileal DAPA, mg/d	75.62	69.85	50.48	55.38	48.15	71.25	7.205
Fecal DAPA, mg/d	212.70[b]	219.92[b]	196.81[b]	187.10[b]	174.13[b]	110.70[c]	11.462
Ileal Bacterial N, g/d	2.86	2.65	1.91	2.10	1.82	2.70	0.273
Fecal Bacterial N, g/d	7.33[b]	7.58[b]	6.79[b]	6.45[b]	6.00[b]	3.82[c]	0.395
Total ileal N, g/d	13.42[bc]	14.56[b]	13.85[bc]	12.12[c]	12.23[c]	8.14[d]	0.509
Total fecal N, g/d	8.91[b]	9.17[b]	8.40[b]	7.48[c]	6.88[c]	4.13[d]	0.281
Ileal BN/TN[e] (%)	21.71[c]	18.22[c]	14.00[c]	16.96[c]	14.91[c]	34.04[b]	2.171
Fecal BN/TN (%)	82.04[c]	82.32[c]	80.17[c]	86.52[c]	87.71[c]	92.77[b]	3.960

[a] Standard error of the mean

[b,c,d] Means in the same row with different superscript letters differ ($P < .05$)

[e] Bacterial Nitrogen/Total Nitrogen

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

A. Discussion

Cereal grains serve as major sources of both energy and protein (amino acids) in diets for swine. The maturity of the digestive tract of growing and finishing pigs allows for efficient digestion of carbohydrates and protein in most cereals. However, there is a scarcity of information on the digestibility of carbohydrate and amino acids in young pigs, in which the digestive system is not yet fully matured (Bengala-Freire et al., 1988).

Commonly used cereals in diets for young pigs include wheat and corn. Limited amounts of barley are sometimes included. The development of hulless barley (HB) with a higher digestible energy content (compared to hulled barley) may result in increased use of barley by young pigs.

Different processing methods can be employed to improve the digestibility of grains, thereby overcoming in part the immaturity of the digestive system of the young pig. These processing methods include extrusion, cooking, boiling and micronization. Heat treatment is involved in most processing methods which may have both beneficial and undesirable effects. Beneficial effects include destruction of antinutritional factors and gelatinization of starch. On the other hand, the Maillard reaction between protein and sugars reduces the nutritive value.

There is a scarcity of information on the effect of micronization on energy and amino

acid digestibility in cereal grains. Micronization is a relatively new process that is used more and more in the feed and food industry. Micronization is a short time high temperature process using moisture, heat and mechanical pressure to achieve conditions essential for optimum cooking and starch gelatinization.

Therefore, studies were carried out to determine the effect of micronization on energy, starch and amino acid digestibility in young pigs. It is important to point out that the ileal analysis method was used to determine amino acid digestibility rather than the fecal method because of the modifying influence of the microflora in the large intestine (Sauer and Ozimek, 1986). It is also important to stress that the difference method was used. As was shown by Fan and Sauer (1995), amino acid digestibilities in ingredients low in amino acid content, which include wheat and barley, should be determined with the difference rather than direct method. The direct method will underestimate digestibility values.

Micronization improveD the apparent ileal digestibilities of most amino acids, both in HB and wheat. In HB, of the indispensable amino acids, the differences ranged from 5.3 to 10.0 percentage units. In wheat, these ranged from 2.2 to 12.2 percentage units. Lysine is the first-limiting amino acid in both HB and wheat. Micronization of HB increased the lysine digestibility from 56.1 to 63.5% The difference, however, was not significant at $P < .05$. In wheat the lysine digestibility was increased ($P < .05$) from 57.6 to 69.7%. Indirectly, these results show that the Maillard reaction did not occur during micronization. Micronization also increased ($P < .05$) the ileal digestibility of starch; from 79.0 to 97.3% in HB and from 93.1 to 99.3% in wheat. In fact, micronization resulted in an increase in digestion and absorption of energy in the small intestine and in a decrease of microbial fermentation of

energy in the large intestine. This shift in disappearance of energy from the large to the small intestine should also result in an improvement in the efficiency of energy utilization as was shown by Just et al. (1983).

In addition to digestibility studies, it will be of interest to carry out performance studies, in which feed intake (reflecting palatability), average daily gain and feed conversion efficiency are measured. The question remains if the improvements in digestibility are sufficient to bring about improvements in performance. Furthermore, it may be of interest to determine the effect of micronization of cereal grains on digestibility in older pigs. However, it is doubtful that there will be an increase in the ileal digestibility of starch and a shift in disappearance of energy from the large to the small intestine. Several studies with older pigs have shown that the ileal digestibility of starch in wheat and wheat by-products approaches 100% (Chapter 4; Lin et al., 1987), and in barley the value is above 98.5% (Lin et al., 1987).

Other studies were carried out with wheat shorts. The greatest problem associated with the use of wheat shorts in diets for swine is the lack of uniformity which results from different proportions of endosperm and bran. Five samples of wheat shorts differing in fiber content were created for these studies. These five samples, hereafter referred to as wheat fractions, were made up of different proportions of bran, flour and shorts which provided a wide range in the content of neutral-detergent fiber (NDF). The NDF content in the wheat fractions ranged from 29.5 to 42.3%. The ileal amino acid digestibilities were determined with the difference method (Fan and Sauer, 1995). Simple linear relationships were established between the apparent ileal amino acid digestibilities and the NDF content in the

wheat fractions. For most amino acids, there were significant ($P < .05$) negative correlations between the apparent ileal amino acid digestibilities and the NDF content in the wheat fractions. The regression equations developed in this study can therefore be used to predict the apparent ileal amino acid digestibilities in wheat shorts which is of practical importance for efficient use of this ingredient in diet formulation. Thereafter, studies were initiated to determine the apparent ileal digestibilities of amino acids associated with the NDF fraction in wheat shorts. As was postulated by Shah et al. (1982) and Bjergegaard et al. (1991) amino acids associated with the NDF fraction are likely to be of low digestibility because the digestive enzymes have limited access. The average digestibilities of the indispensable amino acids (over all diets) were relatively low and ranged from 48.2% for isoleucine to 66.8% for arginine. It is of interest to point out that these digestibility values represent true rather than apparent values. The apparent values will be lower as dietary fiber per sé may affect the amount of endogenous amino acids in ileal digesta. Further studies should be carried out on this topic with other feed ingredients that have a relatively high content of NDF.

As part of these studies it was observed that the ileal digestibilities of NDF ranged from 16.2 to 18%. As pigs do not produce enzymes that digest NDF, the disappearance of NDF in the small intestine must result from bacterial fermentation. To follow up this observation, studies were carried out to determine the content of bacterial nitrogen in ileal digesta. The contribution of bacterial to total nitrogen in ileal digesta was considerable and ranged from 14.0 to 34.0%. In quantitative terms, however, there were no differences ($P > .05$) in the output of bacterial nitrogen in ileal digesta among the experimental diets. There is a need to carry out more studies on the effect of bacterial nitrogen (protein) on the

interpretation of amino acid digestibility values as these are determined with the ileal analysis method. For example, to which extent is bacterial protein derived from amino acids directly or from urea.

Finally, studies were carried out to determine the effect of dietary fiber on the output of mucin in ileal digesta. There were no differences ($P > .05$) in mucin outputs among the experimental diets, which differed considerably in NDF content. The result of these studies throw some doubt on the general belief that dietary fiber, depending on its level in the diet, effects the synthesis and/or degradation of mucins.

B. Conclusions

In summary, the following conclusions can be drawn:

1. Micronization of HB and wheat improved the digestibility of energy and the ileal digestibilities of most of the amino acids in young pigs.

2. Micronization of HB and wheat shifted the disappearance of starch from the large to the small intestine which should result in an improvement in the efficiency of energy utilization in young pigs.

3. There were large differences in the apparent ileal digestibilities of amino acids among the wheat fractions. Differences in NDF content were , in part, responsible.

4. A large proportion of CP and amino acids in the wheat fractions were associated with

NDF. The apparent ileal digestibilities of CP and amino acids associated with NDF were relatively low.

5. There were no differences ($P > .05$) in the output of mucin at the distal ileum among the experimental diets, which differed considerably in dietary fiber content.

6. There were no differences ($P > .05$) in the output of bacterial nitrogen in ileal digesta. However, there were differences ($P < .05$) in the contribution of bacterial to total nitrogen in ileal digesta, ranging from 14.0 to 34.0%.

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